A STUDY ON SERUM VITAMIN D LEVELS

IN

ACUTE ISCHAEMIC STROKE

A Dissertation Submitted to

THE TAMILNADU DR. M.G.R MEDICAL UNIVERSITY

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For the Award of the Degree of

M.D. (GENERAL MEDICINE) - BRANCH – I

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BONAFIDE CERTIFICATE

This is to certify that " **A STUDY ON SERUM VITAMIN D LEVELS IN ACUTE ISCHAEMIC STROKE"** is a bonafide work done by

Dr. BALA VIGNESH.S , Post graduate student, Department of General Medicine, Kilpauk Medical College, Chennai-10, under my guidance and supervision in partial fulfilment of rules and regulations of the Tamil Nadu Dr. M.G.R Medical University, for the award of M.D. Degree Branch I (General Medicine) during the academic period from May 2012 to April 2015.

Prof. Dr.R.Sabaratnavel M.D.
Professor and HOD,
Department of Medicine,
Kilpauk Medical College,
Chennai

Prof. Dr.T.Ravindran M.D., DNB.
Professor and Unit Chief,
Department of Medicine,
Kilpauk Medical College,
Chennai

Prof. Dr.N.Gunasekaran M.D., D.T.C.D
The DEAN
Govt.Kilpauk Medical College
Chennai - 600 010

DECLARATION

I solemnly declare that this dissertation " **A STUDY ON SERUM VITAMIN D LEVELS IN ACUTE ISCHAEMIC STROKE "** was prepared by me at Government Kilpauk Medical College and Hospital, Chennai, under the guidance and supervision of **Dr. T. RavindranM.D., DNB.,** Professor and Unit Chief, Department of Internal Medicine, Government Kilpauk Medical College and Hospital, Chennai.

This dissertation is submitted to **The Tamil Nadu Dr. M.G.R. Medical University, Chennai** in partial fulfilment of the University regulations for the award of the degree of **M.D. Branch I (General Medicine)**.

Place: Chennai-10 Dr.S.BALA VIGNESH

Date:

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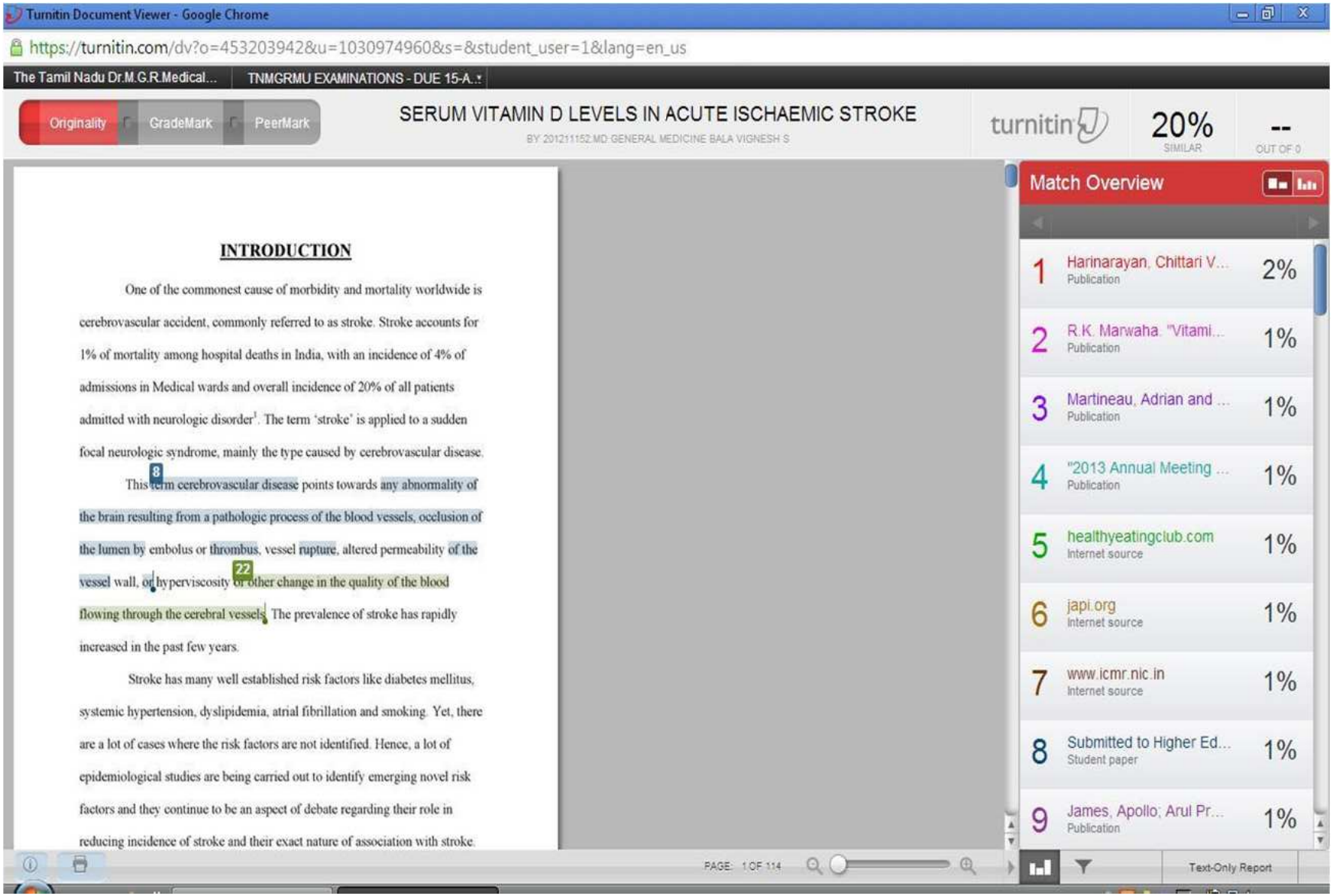
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INTRODUCTION
One of the commonest cause of morbidity and mortality worldwide is cerebrovascular accident, commonly referred to as stroke. Stroke accounts for 1% of mortality among hospital deaths in India, with an incidence of 4% of admissions in Medical wards and overall incidence of 20% of all patients admitted with neurologic disorder[1]. The term 'stroke' is applied to a sudden focal neurologic syndrome, mainly the type caused by cerebrovascular disease.
This term cerebrovascular disease points towards any abnormality of the brain resulting from a pathologic process of the blood vessels, occlusion of the lumen by embolus or thrombus, vessel rupture, altered permeability of the vessel wall, or hyperviscosity or other change in the quality of the blood flowing through the cerebral vessels. The prevalence of stroke has rapidly increased in the past few years.
Stroke has many well established risk factors like diabetes mellitus, systemic hypertension, dyslipidemia, atrial fibrillation and smoking. Yet, there are a lot of cases where the risk factors are not identified. Hence, a lot of epidemiological studies are being carried out to identify emerging novel risk factors and they continue to be an aspect of debate regarding their role in reducing incidence of stroke and their exact nature of association with stroke.
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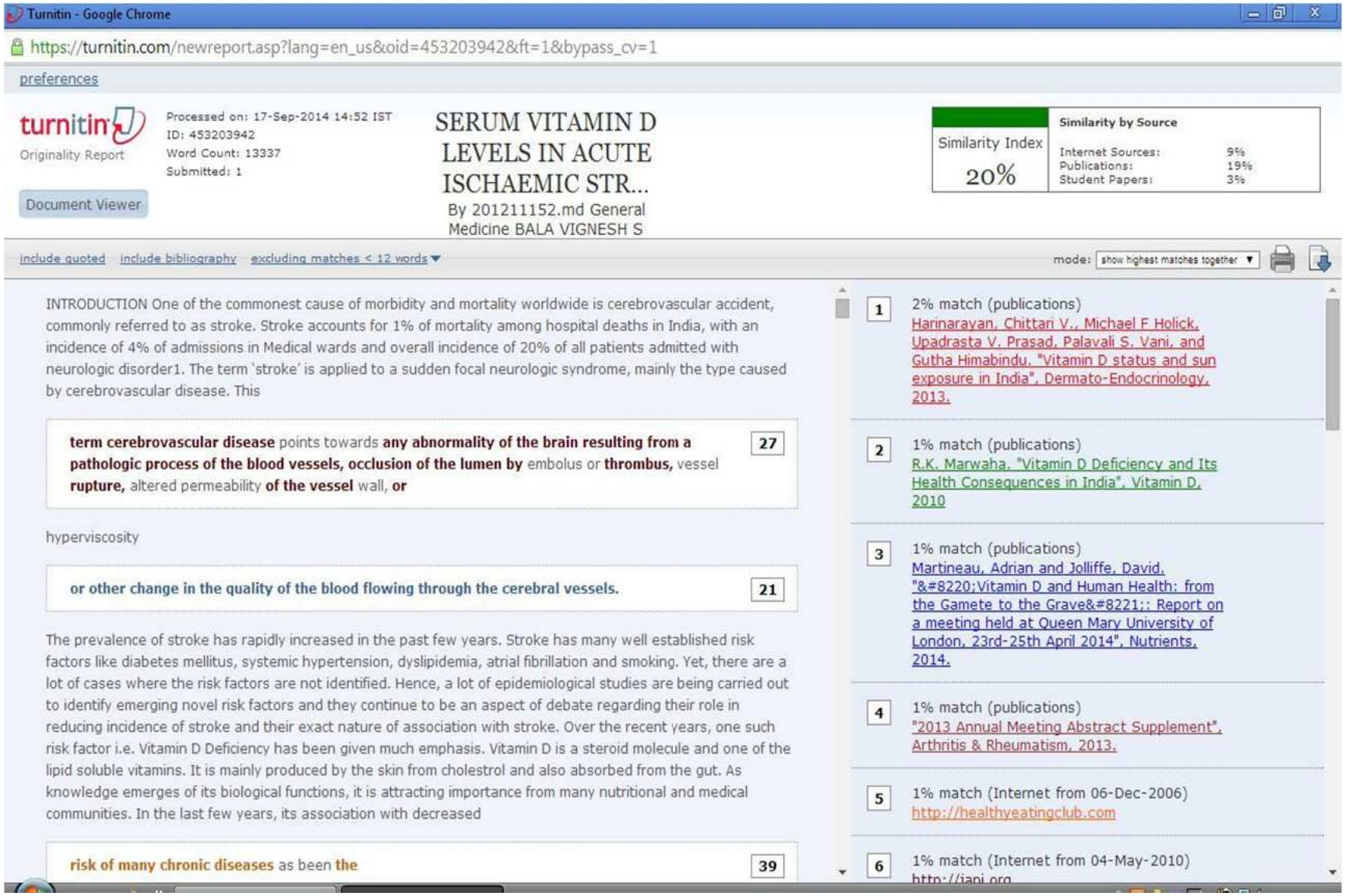
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The prevalence of stroke has rapidly increased in the past few years. Stroke has many well established risk factors like diabetes mellitus, systemic hypertension, dyslipidemia, atrial fibrillation and smoking. Yet, there are a lot of cases where the risk factors are not identified. Hence, a lot of epidemiological studies are being carried out to identify emerging novel risk factors and they continue to be an aspect of debate regarding their role in reducing incidence of stroke and their exact nature of association with stroke. Over the recent years, one such risk factor i.e. Vitamin D Deficiency has been given much emphasis. Vitamin D is a steroid molecule and one of the lipid soluble vitamins. It is mainly produced by the skin from cholestrol and also absorbed from the gut. As knowledge emerges of its biological functions, it is attracting importance from many nutritional and medical communities. In the last few years, its association with decreased
risk of many chronic diseases as been the
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ABSTRACT

BACKGROUND / OBJECTIVES:

Stroke has many well established risk factors like diabetes mellitus, systemic hypertension, dyslipidemia, atrial fibrillation and smoking. Vitamin D is being looked upon as one of the latest risk factors. Our aim was to find out the association between Vitamin D and acute ischaemic stroke, and the effect of parameters like age, gender, obesity and dyslipidemia on Vitamin D levels.

MATERIALS AND METHODOLOGY:

Cross sectional study of 100 subjects – 50 patients with acute ischaemic stroke and 50 age and sex matches controls without stroke, studied consecutively at Kilpauk Medical College Hospital, Chennai. Serum 25 Hydroxy Vitamin D levels were measured along with Body Mass Index, LDL cholestrol, HDL cholestrol, Triglycerides and Total Cholestrol.

RESULTS:

Stroke patients had significant Vitamin D deficiency (mean-13.48ng/ml, p value<0.01) when compared to controls (mean 23.03ng/ml). Gender variation and smoking did not affect the Vitamin D levels. Vitamin D was found to be significantly lower in cases with age less than 40yrs (p-0.046). Vitamin D levels were not affected Body Mass Index or lipid levels.

CONCLUSION:

Acute ischaemic stroke patients have significant Vitamin D deficiency. Vitamin D deficiency could be the causative factor for stroke in these patients. Correction of Vitamin D status can prevent stroke in Vitamin D deficient individuals.

KEYWORDS

25 hydroxy Vitamin D, Gender, Age, Body Mass Index, Dyslipidemia, Stroke

INTRODUCTION

One of the commonest causes of morbidity and mortality worldwide is cerebrovascular accident, commonly referred to as stroke. Stroke accounts for 1% of mortality among hospital deaths in India, with an incidence of 4% of admissions in Medical wards and overall incidence of 20% of all patients admitted with neurologic disorder[1]. The term 'stroke' is applied to a sudden focal neurologic syndrome, mainly the type caused by cerebrovascular disease.

This term cerebrovascular disease points towards any abnormality of the brain resulting from a pathologic process of the blood vessels, occlusion of the lumen by embolus or thrombus, vessel rupture, altered permeability of the vessel wall, or hyperviscosity or other change in the quality of the blood flowing through the cerebral vessels. The prevalence of stroke has rapidly increased in the past few years.

Stroke has many well established risk factors like diabetes mellitus, systemic hypertension, dyslipidemia, atrial fibrillation and smoking. Yet, there are a lot of cases where the risk factors are not identified. Hence, a lot of epidemiological studies are being carried out to identify emerging novel risk factors and they continue to be an aspect of debate regarding their role in reducing incidence of stroke and their exact nature of association with stroke.

Over the recent years, one such risk factor i.e. Vitamin D Deficiency has been given much emphasis. Vitamin D is a steroid molecule and one of the lipid soluble vitamins. It is mainly produced by the skin from cholestrol and also absorbed from the gut. As knowledge emerges of its biological functions, it is attracting importance from many nutritional and medical communities. In the last few years, its association with decreased risk of many chronic diseases as been the talk of the town.

Vitamin D deficiency is a worldwide health problem. In addition to its well accepted role as a major regulator of calcium and bone metabolism, many studies have shown strong association of hypovitaminosis D with systemic hypertension, coronary artery disease, diabetes mellitus, heart failure, metabolic syndrome, cancer, peipheral artery disease and many autoimmune disorders.

Few worldwide studies have shown association between Vitamin D deficiency and an increased incidence of Cerebrovascular Accident (Stroke). Vitamin D deficiency is postulated to cause endothelial dysfunction.This plays a vital role in the pathogenesis of stroke. Following the discovery of the expression of Vitamin D receptors and 1α hydroxylase in the endothelium of blood vessels, several biological mechanisms that link Vitamin D with stroke and its risk factors have been identified. Vitamin D acts mainly through its role in maintaining gene transcription to prevent cerebrovascular disease and its risk factors.

In spite of the rising proportion of stroke in Asians, only limited data is available on the relationship between Vitamin D and stroke. Since Vitamin D levels are directly measurable and its deficiency can be treated, many trials are being done to assess its association with stroke and to prevent stroke if possible.

A practical time to check 25-hydroxy vitamin D levels would be at the time of an acute ischaemic stroke. Hence this study was designed to assess the vitamin D levels in acute ischaemic stroke and to find out any significant correlation.

AIMS AND OBJECTIVES

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1. The primary aim of the study was to measure the serum 25hydroxy-Vitamin D levels in patients with acute ischaemic stroke and to compare their levels with age and sex matched controls.

2. The secondary objectives were to assess the effect of age, sex, obesity, smoking and dyslipidemia on Vitamin D levels.

REVIEW
OF
LITERATURE

REVIEW OF LITERATURE

STROKE

DEFINITION:

A stroke or cerebrovascular accident is defined as neurological deficit that is abrupt in onset, attributable to a focal neurological cause, lasting more than 24hours.[2] The term 'cerebrovascular disease' indicates any abnormality of the brain resulting from a pathologic process of the blood vessels, including rupture of a vessel, occlusion of the lumen by embolus or thrombus, increased viscosity or an altered permeability of the vessel wall, or any other change in the quality of the blood flowing through the cerebral vessels.

STROKE STATISTICS – GLOBAL SCENARIO

In the recent years, due to the increase in the development of economy and demography, there is a shift towards lifestyle-related chronic non-communicable diseases in the developing and developed countries. In the developing as well as developed countries, one of the causes of serious long term neurological disability is stroke. It also makes an important contribution to morbidity and mortality.

Worldwide, stroke is the third most common cause of death following coronary artery disease and cancer.[3] It is the fourth leading cause of disease burden[3]. More than the sixth decade, the prevalence of stroke is nearly three-fourths more. There is no age limit for stroke to occur. One-fourths of the overall incidence of stroke occurs in people less than 65yrs of age.[4] The stroke risk is doubled for each decade, after the age of 55yrs.[5]

The incidence of stroke in entire world population is 0.22 per 1000 people. According to World Health Organisation (WHO) report, approximately around 15million people are struck by stroke every year. Out of these strokes, higher systemic hypertension contributes to 12.7 million. Out of the 15million people affected by stroke every year,around one third die and another one third have permanent disability.[6] The number of people suffering from stroke in increasing in developing countries, largely due to the fact that systemic hypertension is not being controlled adequately. Smoking and ageing of the population as a whole also contributes significantly.

STROKE – INDIAN SCENARIO:

When compared to few developed countries, where the incidence of stroke as reached a plateau or has even decreased, stroke burden as been increasing in India. In India, ischaemic stroke contributes to around 80% of all strokes.[7] It is estimated that, by 2015, around 1.6 million cases of stroke will be reported annually. One third of these will have permanent disability. WHO has

estimated that by 2050, 80% of all strokes in the world will occur in India and China.[7] Indian studies show that about 10-15% of all strokes occur in people of less than 40yrs of age.

RISK FACTORS FOR STROKE

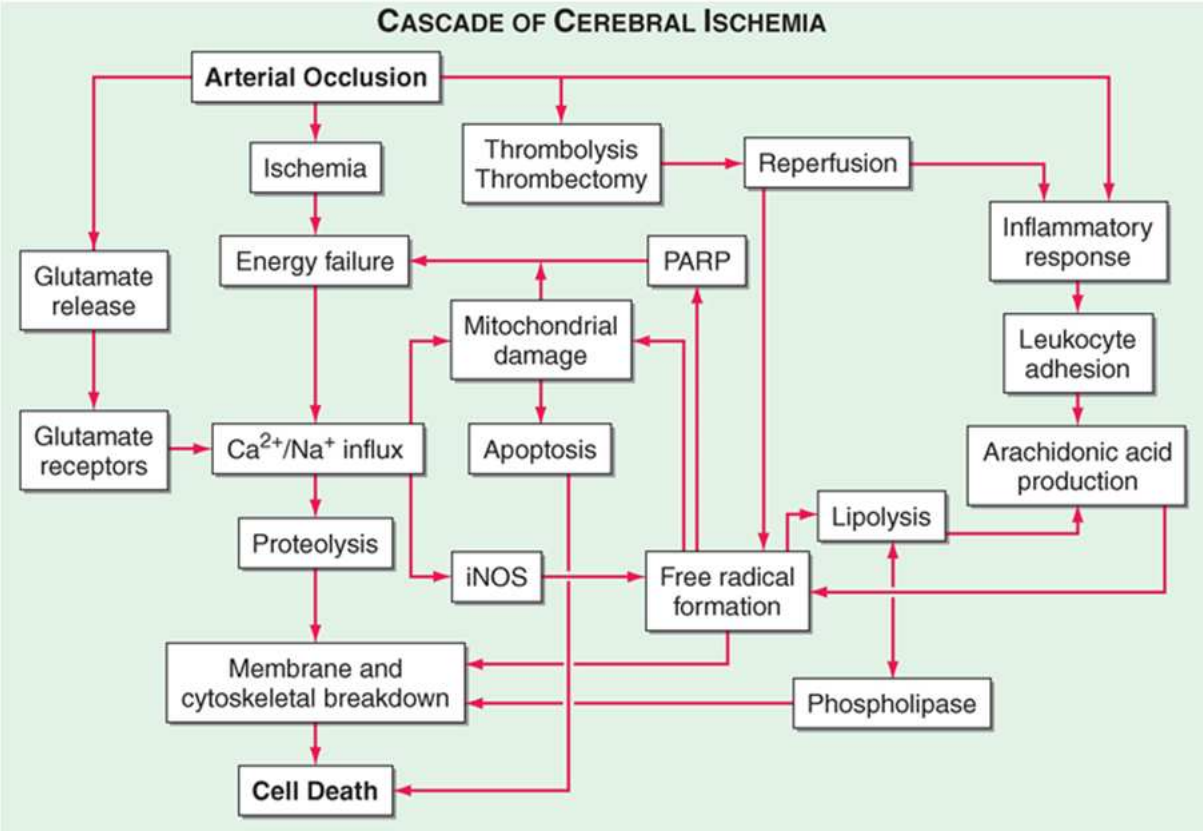
Non- Modifiable risk factors[2]

- Old age
- Male sex
- Post menopausal women
- Type A personality
- Family history
- Genetic factors

Modifiable risk factors

- Diabetes mellitus
- Systemic hypertension
- Dyslipidemia
- Smoking
- Obesity
- Stress
- Sedentary habits

PATHOGENESIS OF STROKE



Considerable progressive development in the understanding of the physiology and pathogenesis of acute ischaemic stroke has taken place in the last two decades. Ischaemic cascade is the series of time dependent neurochemical events that take place after occlusion of the intracranial cerebral vessels. The flow disturbance causes rapid, secondary and delayed effects.

Rapid effects – oxygen depletion, energy failure, terminal depolarization and ion homeostasis failure. It occurs within minutes.

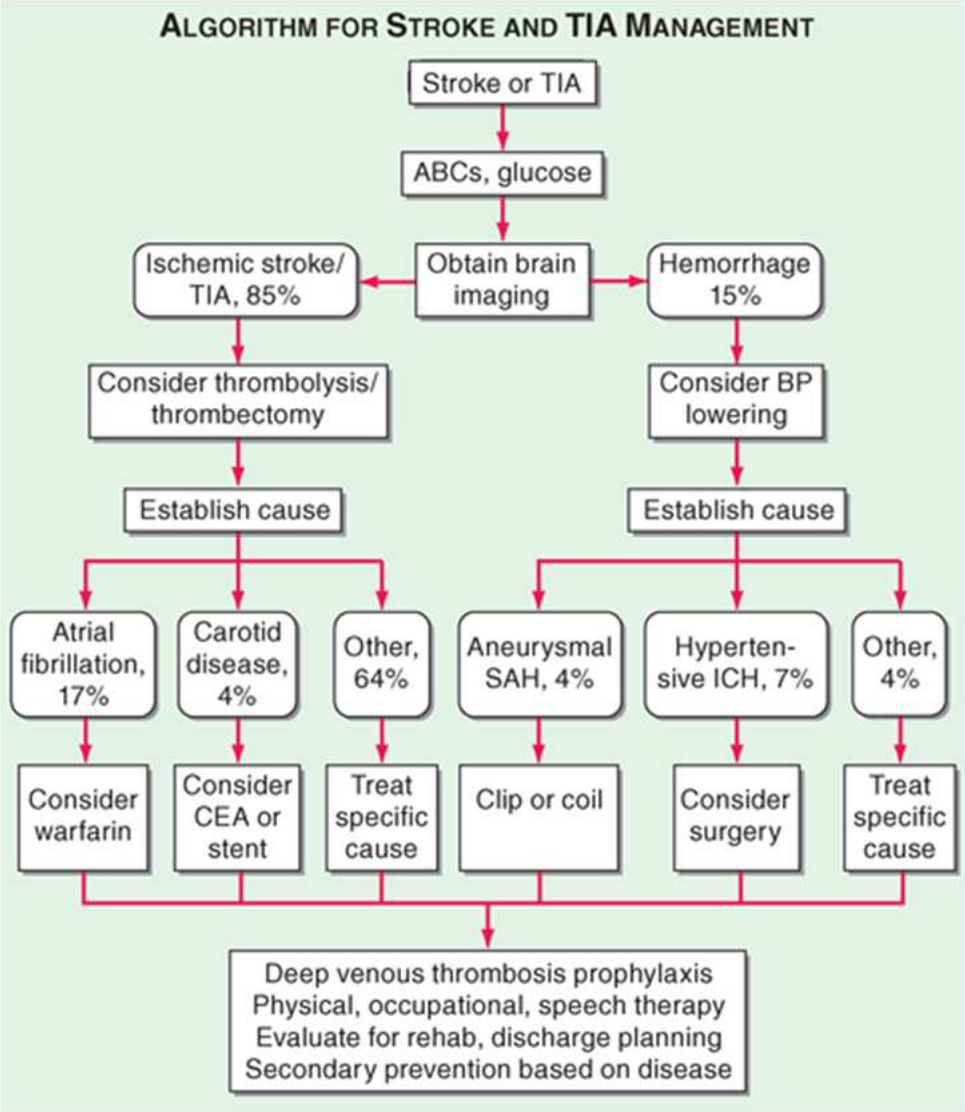
Secondary effects – excitotoxicity, SD-like depolarizations and disturbance of ion homeostasis. It occurs within hours.

Delayed effects – inflammation and apoptosis. It occurs in few days to few weeks of onset of flow disturbance.

The neuropathogenic processes involved in the ischaemic insult are-

- An excitatory aminoacid, glutamate, is the most excessive excitatory neurotransmitter in the brain. It is stored in the presynaptic vesicles. Upon release, it binds to the post synaptic NMDA (N-methyl D-aspartate) receptor.[8]
- Once the reduction of cerebral blood flow commences, abundant release of excitatory neurotransmitters occurs, mainly glutamate, causing excessive activation of the NMDA receptor.
- Once these receptors get activated, there is excessive influx of sodium and calcium ions through the voltage and ligand gated channels.
- The intracellular enzyme systems lead to the induction of-
  i. Free radical production[9]
  ii. Initiation of an inflammatory response which stimulates apoptosis
  iii. Membrane lipid breakdown proteolysis
- Compromise of metabolic functions occurs with expansion of the infarct volume and neurotoxicity over days to weeks.
- Direct microvascular damage and worsening ischaemia occurs as a result of leucocyte and platelet activation.[10]

MANAGEMENT OF STROKE



RECENT TRENDS IN THE MANAGEMENT OF STROKE

Dramatic improvement in the management of ischaemic stroke has been seen in recent times. Therapeutic strategies have been divided into those targeting the nervous system and those targeting the vasculature. Current vascular strategies include recanalisation by clot removal (thrombolysis, intraarterial fibrinolysis, mechanical removal) and prevention of propagation of

clot with aspirin and atorvastatin. Protection to the brain is given by these agents mainly through the hemodynamics rather than the metabolic mechanisms.[11]

INTRAVENOUS THROMBOLYSIS

The use of intravenous thrombolytic therapy in acute ischaemic stroke is strongly time dependent.In the first few minutes of symptom onset, therapeutic yield is maximum. It declines steadily during the first three hours. The 'golden hour' for therapy is the first 60minutes of onset of symptoms. Recanalisation therapy has maximum benefit in this golden hour. Target door to needle time is <60mins. This is achieved in less than one fifth of golden hour-arriving patients. In a typical acute ishaemic stroke, every minute the brain loses 14 billion synapses, 7.5miles myelinated fibres and 1.9million neurons.[12] 1 fewer patient has improved for every 10minute delay in delivery of recombinant tissue plasminogen activator.

The window period for intravenous thrombolysis is 4.5 hours, that is, 4.5 hours from the time of onset of stroke symptoms. Therapeutic window is the time until the area becomes irreversibly damaged.[13] The window period for intravenous thrombolysis has been recently increased from 3 hours to 4.5 hours.[14] However, only a small proportion of patients reach the medical setups within this time frame. So, the majot target for management of stroke is in

protecting the brain from ischaemic damage and preventing the occurence of stroke.

Current neural strategies for treating acute ischaemic stroke include acute neuroprotection and promotion of brain plasticity. Various neuroprotective agents like calcium antagonists, free radical scavengers, glycine antagonists, etc, which intervene in one of the steps of ischaemic cell injury have been used in the past for treating ischaemic stroke.[15] But they were either too toxic to humans or ineffective.

THE NEED FOR NEUROPROTECTION:

Once ischaemic damage occurs, the neurological insult spreads from the core of the infarct. The maximal size of the infarct is produced by the excitotoxic injury which continues beyond 48 hours. For the next 72 hours, the local cerebral perfusion and autoregulation are disturbed.[16] In most cases, within 72-96 hours, collateral vessels develop and the damaged areas get reperfused. To some extent, the regional blood flow abnormalities also tend to resolve.[17]

The areas of ischaemia which will transform into an infarct cannot be identified even by Positron Emission Tomography (PET) imaging, which can usually distinguish ischaemia from infarct. It was found that within 9 hours of the insult, blood flow was reduced locally in 100% of patients and within 4

days, it was reduced to 30%. Even upto 48 hours after stroke, this ischaemic but viable tissue can be found.

The time window for thrombolysis is relatively short because of the high hemorrhagic complications when reperfusion therapy is done at later stages. So there is a rationale for initiating neuroprotective measures in order to prevent the ongoing cerebral ischaemia. This also salvages the viable ischaemic tissue at a time when cerebral autoregulation is deranged.[18]

CLASSIFICATION OF NEUROPROTECTIVE AGENTS[8]

1. Modulators of Calcium Influx
2. Modulators of Excitatory Amino Acids
3. Metabolic Activators
4. Inhibitors of Leukocyte Adhesion
5. Anti edema agents
6. Promotors of Membrane Repair
7. Free Radical Scavengers and Anti-Oxidants

The most promising neuroprotective agents are the therapeutic hypothermia, hyperacute magnesium therapy, high dose human albumin, GABA agonists, calcium channel blockers, glutamate antagonists, down-regulators of the nitric oxide signal transduction, free radical scavengers and antioxidants. These are the ones that have been most extensively studied.[19]

In recent times, many clinical trials conducted have proved the neuroprotection offered by Vitamin D and its role in preventing ischaemic stroke.

PROGNOSIS:

The most common cause of neurological disability in the world is stroke. Patients with stroke have a worse morbidity than any type of cancer. Approximately, 75% of the patients affected with stroke become functionally dependent.[20] The Indian Council of Medical Research (ICMR) estimated that stroke contributes for 72 % of Disability Adjusted Life Years (DALYS) and 41% of deaths among the non-communicable diseases (NCDs).[20] Many problems like seizures, fractures, falls, dementia and depression occur secondary to stroke. So, stroke patients have residual disabilities which make them physically dependent, causing enormous socio-economic impact on health care institutions, individuals and families.

ISCHAEMIC PENUMBRA

It is defined as that region of the ischaemic zone that is potentially salvageable.[13] Occlusion of the middle cerebral artery causes the blood flow of the core region to reduce below 10ml/100gm/min, which causes rapid necrosis of this region. The region surrounding the core region is the ischaemic

penumbra, where a blood supply of 10-20ml/100gm/min is supported by the collaterals.[9]

Within this ischaemic penumbra, majority of the ischaemic cascade takes place and that too within the first two hours of the onset of focal ischaemia.[21] The ischaemic penumbra is functionally impaired tissue. It is viable upto 48hours after the onset of stroke. The penumbra can be saved by timely intervention by reperfusion with thrombolytics or attenuating the ischaemic cascade with neuroprotective agents. Otherwise, the metabolic and neurochemical consequences of ischaemia causes the penumbra tissue to become necrosed.[22]

The main aim of treatment is in improving the disparity between the energy supply and demand of the brain, thereby reducing the neuronal damage. This is done by supplying alternative metabolic substrates, restoring local blood flow, by reducing the neuronal metabolism, protecting against the toxic effects of the ischaemic cascade. Thus, improving the overall metabolic environment.[10]

Only a few treatment modalities are available for saving the ischaemic penumbra region. Current therapies include intravenous thrombolysis with tissue plasminogen activator, intraarterial fibrinolysis, mechanical removal, aspirin and moderate hypothermia (33 degree celsius) for cardiac arrest and decompressive hemicraniectomy for ischaemic stroke.

STROKE MIMICS

- Seizures
- Migraine
- Hypoglycaemia
- Syncope
- Hypoglycaemia
- Herpes simplex encephalitis
- Central nervous system tumors
- Drug overdose
- Subdural hematoma
- Conversion syndrome

VITAMIN D

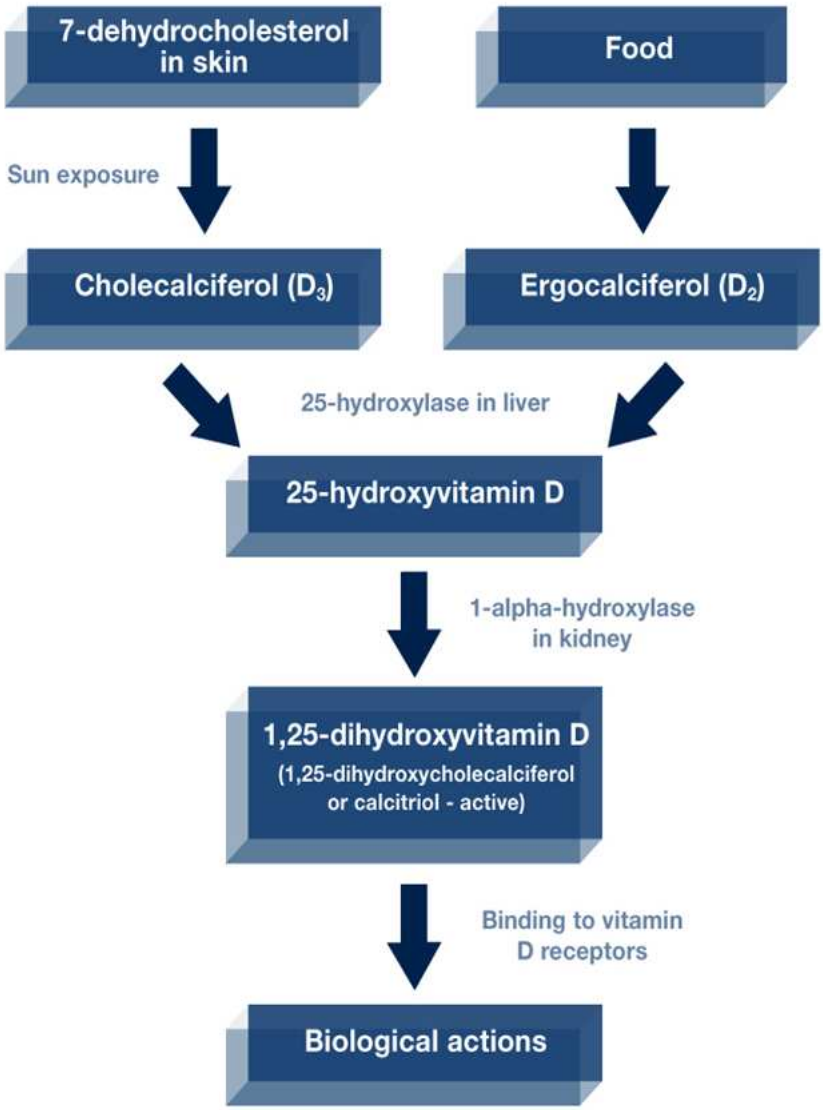
Vitamin D has been traditionally known as the 'sunshine vitamin' or 'anti ricketic factor'. It is unique because it is the only endogenously synthesized vitamin that also acts as a hormone. Besides its main role in calcium homeostasis and bone metabolism, the vitamin D endocrine system is found to have a wide range of fundamental biologic functions in inhibition of cell growth, immunomodulation and cell differentiation.[23-25]

SYNTHESIS AND METABOLISM OF VITAMIN D

Vitamin D is a secosteroid. It is one of the fat soluble vitamins. The main precursors of Vitamin D are Vitamin D2 (ergocalciferol) and Vitamin D3 (cholecalciferol).[26]

In the skin, 7-dehydrocholesterol is converted to previtamin D3. This is done by exposure to Ultraviolet B rays (290-320nm wavelength). This is then converted to Vitamin D. Major portion of circulating 25 hydroxy Vitamin D (40-50%) is derived from skin. Vitamin D2 is obtained from diet and also formed in plants.

In the liver, 25 hydroxylase enzyme acts on Vitamin D2 and D3 and converts them into 25-hydroxy vitamin D (calcidiol). In the kidneys, 1Ω hydroxylase acts on 25-hydroxy vitamin D to form biologically active 1,25-dihydroxy Vitamin D (calcitriol).



MECHANISM OF ACTION

The component circulating in blood is 25 hydroxy Vitamin D. The active component, 1,25hydroxy vitamin D (Calcitriol) is transported in the blood to many target organs by Vitamin D Binding Protein (VDBP). Calcitriol acts

through the Vitamin D receptors (VDR). Vitamin D receptors belong to the nuclear receptor superfamily. After activation, the Vitamin D receptor dimerizes with Retinoid X Receptor (RXR) and binds to Vitamin D responsive elements which regulate the transcription of various genes in the target cells.

The control of transcription requires recruitment of additional co-regulators that may be either stimulatory(co-activators) or inhibitory (co-suppressors). Certain genes are selective for their co-regulators. Inhibitors of transcription and translation can block these genomic responses.

Around 37 different cell types and more than 500 genes have been identified and found to express Vitamin D receptor. Almost all cells in the body have Vitamin D receptor. Vitamin D regulates around 10% of the human genome. Vitamin D has so many pleiotropic actions because of this ubiquitous nature of Vitamin D receptor.

CHEMICAL MESSENGER

1,25 dihydroxy vitamin D serves as a chemical messenger that transmits rapid responses and signals(eg. opening of ion channels). A variety of receptors mediate rapid responses. These receptors are associated with plasma membrane or its caveolae components. Caveolae are flask shaped membrane invaginations that are rich in cholestrol and sphingolipids.

Examples of rapid responses include secretion of insulin by pancreatic beta cells, intestinal absorption of calcium, rapid migration of endothelial cells and opening of voltage gated calcium and chloride channels of osteoblasts. Individual tissues produce their own Vitamin D3 in a tissue specific fashion. This ability explains how Vitamin D regulates selective functions in many tissues.

SOURCES OF VITAMIN D

SUNLIGHT

Sunlight is the main natural source of ultraviolet B rays. 20-30 minutes of sunlight exposure, 2-3 times a week, between 10am and 3pm is considered sufficient. Vitamin D excess due to UVB exposure doesnot occur because excess UVB rays convert Vitamin D3 into tachysterol and lumisterol, which are inactive metabolites. 3000IU of Vitamin D3 is provided by 0.5MED of UVB rays.

UVB rays are reduced by 60% by shade and severe pollution[27]. These rays donot penetrate glass, hence sunlight exposure indoors is of no use.[27] More active Vitamin D is provided by sunlight than any other source.

FOOD[27]

Cod liver oil and oily fish are important sources of Vitamin D3. Generally vegetables are a poor source and hence food fortification programmes play a significant role in vegetarian diet.

Cod liver oil (1 tsp)	400-1000 IU of Vitamin D3
Salmon Fish (100gm)	600-1000 IU of Vitamin D3
Tuna Fish (100 gm)	230 IU of Vitamin D3
Mackerel Fish (100gm)	250 IU of Vitamin D3
Egg yolk	20 IU of Vitamin D3

SUPPLEMENTS

Vitamin D3 (cholecalciferol) and Vitamin D2 (ergocalciferol) are available as supplements. Among the two, most effective is Vitamin D3.

VITAMIN D DEFICIENCY (HYPOVITAMINOSIS D)

There are many guidelines that define cut off values to assess Vitamin D deficiency. Recent consensus suggests that serum 25-hydroxy Vitamin D > 30ng/ml as the cut off value because this is the lower limit (threshold) at which optimum calcium absorption occurs and parathormone secretion is induced.[28]

25 HYDROXY VITAMIN D – REFERENCE RANGE[29]

- <10 ng/ml – Vitamin D Deficiency
- 10.1-30 ng/ml – Vitamin D Insufficiency
- 30.1-100 ng/ml – Normal value (sufficient)
- >100 ng/ml – Vitamin D intoxication

CAUSES OF VITAMIN D DEFICIENCY

REDUCED SKIN SYNTHESIS

- Skin pigmentation, sunscreen use, obesity and ageing reduce UVB related synthesis of Vitamin D from skin.
- Latitude, time of the day and season also determine the production of Vitamin D from skin.
- Elderly persons spend less time outdoors and have reduced 7- dehydrocholestrol as well.
- More melanin is present in dark skinned individuals which competes with 7 dehydrocholestrol for absorption of UVB rays.
- UV rays will be blocked by sunscreens with a sun protection factor (SPF) of 8 or more.

INADEQUATE DIETARY INTAKE

- Elderly and children are especially susceptible.
- Human breast milk is a poor source of Vitamin D, hence infants are also more prone for Vitamin D deficiency.

REDUCED BIOAVAILABILITY

- Malabsorption disorders[30]
- Liver Failure – impaired synthesis of 25 hydroxy Vitamin D
- Renal Failure – impaired 1α hydroxylase activity
- Obesity – Vitamin D is sequestrated in body fat
- Drug interactions – Glucocorticoids, Rifampicin, Antiepileptics

REDUCED SYNTHESIS OF ACTIVE VITAMIN D

- Hyperphosphatemia increases fibroblast growth factor (FGF-23) which decreases 1α hydroxylase activity[31].
- Chronic kidney disease

INCREASED LOSS OF 25HYDROXY VITAMIN D

- Nephrotic syndrome – urinary loss of 25 hydroxy Vitamin D bound to Vitamin D binding protein.

INHERITED DISORDERS

- Vitamin D dependent rickets
- Hypophosphatemic rickets – autosomal dominant and X-linked
- Vitamin D resistant rickets

ACQUIRED DISORDERS

- Hyperthyroidism – increased metabolism of 25 hydroxy Vitamin D
- Primary hyperparathyroidism
- Tumor induced osteomalacia – tumor secretion of FGF 23
- Granulomatous diseases like tuberculosis, sarcoidosis and few types of lymphomas.

PREVALENCE OF VITAMIN D DEFICIENCY

GLOBAL BURDEN

Worldwide, one billion people are estimated to be Vitamin D deficient. The World Health Organisation, after a meta analysis, stated that 50-80 % of the population is Vitamin D insufficient.[29] Vitamin D insufficiency constitutes 9.4% of the global disease burden.[27] Worldwide, approximately 3.3billion DALYs are lost from bone disease due to Vitamin D deficiency.[27]

VITAMIN D DEFICIENCY – INDIAN SCENARIO[32]

Previously , there was a general disbelief that because India is near the equator and receives ample sunshine, Vitamin D deficiency was not prevalent in India. But recent data has proved approximately 50-90% prevalence of Vitamin D deficiency.[32]

A study done by **Goswami et al in New Delhi[33]**, India in the year 2000 showed that 90% of people in New Delhi are Vitamin D insufficient. Subsequent studies in urban and rural areas have shown widespread Vitamin D deficiency in Indians, irrespective of age and sex.[32]

In one of the recent studies, **Ritu G et al[37]** showed that 70% of Indians have Vitamin D deficiency. They suggested food fortification as a must in all parts of India.

Several factors contribute to this high prevalence –

1. Calcium and Vitamin D are low in diet, especially in vegetarians.
2. Less time spent outdoors as a result of urbanization.
3. High fibre content, phytates and phosphates in diet.
4. Outdoor exposure to sunlight is reduced by humid and sultry climate.
5. Darker skin pigmentation
6. Ultraviolet rays are hampered by increased pollution.
7. Muslim customs like Burqa/Pardah.

8. Lack of vitamin D food fortification programmes.
9. Vitamin D deficiency is aggravated in the mother and foetus by repeated and unspaced pregnancies.
10. Skin disorders, liver, kidney, alcoholics, genetic factors, inflammatory rheumatological conditions and malabsorption disorders can lead to Vitamin D deficiency.

VITAMIN D DEFICIENCY AND CLINICAL DISEASE STATES

SYMPTOMS OF VITAMIN D DEFICIENCY

Previously,Vitamin D deficiency was thought to be usually asymptomatic but now it is accepted as an important global health problem. It is associated with multiple non-specific complaints such as[32]

- Fatigue, generalised myalgia and weakness, muscle cramps
- Weight gain, sleeplessness
- Joint pain
- Headache
- Poor concentration

DISEASES ASSOCIATED WITH VITAMIN D DEFICIENCY

- Rickets and osteomalacia
- Malignancy
- Osteoporosis and osteopenia
- Systemic hypertension
- Diabetes mellitus
- Cardiovascular diseases
- Obesity
- Metabolic syndrome
- Autoimmune diseases, Multiple sclerosis
- Parkinsons disease, Alzheimers disease
- Rheumatoid arthritis, Osteoarthritis
- Fibromyalgia, Chronic fatigue syndrome
- Depression, Seasonal affect disorder
- **Cerebrovascular accident (stroke)**

VITAMIN D REQUIREMENTS AND SUPPLEMENTATION

- The FAO/WHO Expert Consultation states that the most physiologically relevant and efficient way of acquiring vitamin D, in most locations in the world around the equator (between latitudes 42 N and 42 S) is to synthesize it endogenously from skin from 7-dehydrocholesterol present

in the subcutaneous fat through a minimum of 30 minutes of skin exposure (without sunscreen) of the arms and face to the mid-day sun.[34]

- Vitamin D synthesized in the skin lasts two times longer in the body as compared to supplemental/ ingested doses. It has been concluded from the experimental data that exposure of the body in a bathing suit (almost 100% of body surface area) to sunlight that causes slight pinkness of the skin (1 MED -minimal erythemal dose) is equivalent to ingesting approximately 20,000 IU of vitamin D orally. Therefore, exposure of 6% of the body to 1 MED is equivalent to taking about 600 and 1,000 IU of vitamin D.[35]
- Applying the rule of nines Burns chart, exposure of both forearms and the face is equivalent to exposing 12% of body surface area. For Caucasian skin (type 2 or 3), exposing the face, arms and legs for a period equal to 25% of the time that it would take to cause 1 MED, two to three times a week can meet the body's vitamin D requirement while minimizing sun damage (" Holick's rule").[36]
- Asians have darker skin (type V) and therefore, with the same amount of MED, they would require a longer duration of sun exposure than their light-skinned counterparts to synthesis comparable amounts of vitamin D.[35] The time required to obtain the recommended UV dose for adequate vitamin D synthesis is "1 Standard Vitamin D Dose" (SDD). Throughout the year 1 SDD for skin type V (Asians) is 10-45 minutes at solar noon,

with longer durations in winter. SDD for skin types is collected on MED. Clouds, aerosols and dense ozone can reduce vitamin D synthesis and force “Vitamin D winter” even at the equator. India is located at between 8.4 and 37.6°N.

- In a study from **South India (Tirupati)**[38-40] using ‘in vitro’ ampoule model with precursors of Vitamin D (7 Dehydrocholesterol), when exposed to sunlight, converted to active vitamin D best between 11 a.m. to 2 p.m (mid-day sun) . The median percentage conversion of 7-DHC to previtamin D and its photoproducts and percentage of previtamin 3D and vitamin D formed were 11.5% and 10.2%, respectively at a solar zenith angle of 36.8° at 12:30 p.m. From the various studies in the literature, it would appear that the 25 (OH)D levels in South Indian subjects are relatively higher than in subjects in North India. There is a strong inverse correlation between the 25 (OH)D levels and latitude ($r = -0.48$; $p < 0.0001$), clearly establishing the relationship between closeness to the equator (smaller zenith angle) and natural Vitamin D synthesis .
- **Studies from Pune**[40] (latitude 18.31°N and longitude 73.55 E) have shown that toddlers exposed to sunlight (playing outside) for more than 30 min a day, exposing more than 40% of their body surface area, had a normal vitamin D status (M: 36.6 ng/ml and F: 27.1 ng/ml), three times more than the toddlers who were indoors for most part of the day (M: 12.8 ng/ml and F: 8.4 ng/ml) .

- A **study in toddlers in Delhi slums**[41] (latitude 28.35 N and longitude 71.12 E) demonstrated that those who were exposed to sunlight had better vitamin D levels (~ 25 ng/ml) as compared to those who were not (~ 8ng/ml). Interestingly, authors of this study also identified (albeit retrospectively) that families whose toddlers were exposed to sunlight had been given educational material by the local healthcare workers explaining the benefits of exposure to sunlight .

VITAMIN D AND AGE VARIATION

VITAMIN D STATUS IN ADULTS

Recent studies from India have shown high prevalence of vitamin D deficiency in both rural and urban populations of adults, in north as well as south India. However, the only large population survey[41] of vitamin D and dietary calcium, done by **Harinarayan et al**[39], is from rural and urban south India . It has been shown in population surveys from South India (Tirupati latitude 13.40 N and longitude 77.2 E) that even rural adult agricultural labourers, despite being exposed to sunlight for more than 4 hours with at least 35% of their body surface area exposed to sunlight, still show vitamin D deficiency. No difference of Vitamin D status among the varous age groups was found.

NEWBORNS AND VITAMIN D DEFICIENCY

More than 3000 genes that affect fetal development[42] are induced by Vitamin D and thus a critical role is played by Vitamin D in brain development and function[43]. Normal transcriptional activity in the brain can be ensured by adequate Vitamin D levels in utero.

Vitamin D deficiency is seen in 84% of pregnant women in India, which correlates with reduced serum 25 hydroxy Vitamin D levels in their newborns. Intrauterine development and postnatal skeletal growth were reduced in their off springs.[44] Human breast milk has very little vitamin D and so exclusively breast fed infants have increased risk of rickets. Vitamin D deficiency compromises the skeletal built from birth and continues into childhood, eventually compromising adult height.

VITAMIN D STATUS IN CHILDREN

Studies show that 60-80% of the variability in bone mass is due to genetic factors, with the rest being attributable to nutrition, lifestyle, physical activity and hormonal factors. Approximately 40-50% of total skeletal mass is accumulated during childhood and adolescence. It is during this period that calcium and vitamin D nutrition and non pharmacologic strategies should be adopted to have the maximum impact on peak bone mass.

The mean serum concentrations of 25(OH)D reported in children and adolescents from urban northern India were 11.8 ± 7.2 ng/ml and 13.84 ± 6.97

ng/ml, respectively . These were lower than those reported in children from southern India . The mean 25(OH)Dconcentrations in adolescents in urban and rural Andhra Pradesh were 17 ng/ml and 18 ng/ml, respectively.[41]

An objective evaluation of the association between nutrition and life style clearly revealed a significant correlation between serum 25(OH)D and estimated sun exposure ($r=0.185$, $p<0.001$) and percentage body surface area exposed ($r=0.146$, $p<0.004$) but not socio-economic status, suggesting that life-style related factors contribute significantly to the low vitamin D status of apparently healthy school girls.[41] The functional significance of low serum 25(OH)D in Indian children is reflected in their serum PTH values.

VITAMIN D STATUS IN REPRODUCTIVE AGE GROUP

Available data from population surveys indicate that the Vitamin D status of women in reproductive age groups is uniformly low in both North and South India . Low vitamin D status and low dietary calcium in reproductive-age women, coupled with unplanned and unspaced pregnancies, lead to decrease in bone mineral density and consequent low peak bone mass, rendering these women vulnerable to postmenopausal osteoporotic fractures later in life.

VITAMIN D STATUS IN POSTMENOPAUSAL WOMEN

Evaluation of daily dietary calcium intake, phytate-to-calcium ratio,and bone mineral parameters in South Indian, postmenopausal women (n=164) showed that their dietary intake of calcium was low compared with the Recommended Dietary Allowance for Indians. Around 85% had either

insufficiency or deficiency of 25 hydroxy Vitamin D. Parathormone and serum alkaline phosphatase levels were significantly higher in patients with 25(OH)D deficiency ($p<0.05$) as compared to those with normal 25(OH)D levels. There was a negative correlation between 25(OH)D and Parathormone ($p<0.007$) and Serum Alkaline Phosphatase ($p<0.001$).[45]

The study concluded that the quality of the diet has to be improved, with enrichment/ supplementation of calcium and vitamin D in order to suppress secondary hyperparathyroidism-induced bone loss and risk of fractures in post-menopausal women . In another study of vitamin D status in postmenopausal women from south India, it was found that vitamin D deficiency coexists with low bone mineral density (BMD).[46] This points to the need to document serum 25(OH)D levels in women with low BMD.

Calcium and vitamin D supplementation should form part of therapy in postmenopausal women . Similar findings were reported from studies carried out in North India.

VITAMIN D AND GENDER VARIATION

In a study conducted by **Johnson et al**[47], it was found that there was significant difference in Vitamin D levels between males and females with a mean Vitamin D level of 21ng/ml among males and 22.4ng/ml among females in Europe. The reason for the gender difference could not be ascertained from the study. Generally men are more exposed to sunlight than females. So they

must have more Vitamin D than women. But this study,gave results against the general consensus.

VITAMIN D AND SMOKING

In a study conducted by **Eugenia Cutillas et al[48],** it was found that smoking causes significant reduction in Vitamin D levels. This study was done in Europe in 2009. It is mentioned that smoking reduces Vitamin D levels by reducing the formation of 25 hydroxy vitamin D. Few other speculations have been made but none have been proved.

OBESITY AND VITAMIN D

Obese individuals need 2 to 3 times more vitamin D per day (that is, 3000 to 6000 IU) to compensate for the impairment in ability to maintain 25- hydroxy Vitamin D levels in the blood. With deficiency of dietary calcium, there is an up to five-fold increase in fatty acid synthetase, an enzyme that converts calories into fat. The presence of sufficiently high levels of calcium and adequate vitamin D inhibits the enzyme.

In obese persons, vitamin D supplementation may improve muscle strength, reduce the occurrence of aches and pains, and enable increased physical activity. It may also help in weight reduction and improve insulin metabolism. It is important to remember that drugs used for reducing fat, such as orlistat inhibit not only the absorption of fat but also that of vitamin D.

Studies done by **Carlin et al** in 2006 and **Aasheim et al** in 2008[48], in Northern America have shown a 40% higher incidence of Vitamin D deficiency among people with obesity. Obesity was classified based on the Body Mass Index in all these studies.

VITAMIN D AND STROKE

Pathophysiological mechanisms remain speculative, but several possible biological mechanisms might explain the association of low 25 hydroxy Vitamin D with stroke.

- Lower vitamin D levels can induce brain damage and cognitive and functional impairment.
- Vitamin D deficiency has been associated with morphological brain changes, motor impairments, and memory and learning impairments in animal models.
- Additionally, numerous further studies have indicated that vitamin D deficiency is associated with accelerated bone resorption and reduced bone mineral density in stroke patients.
- In addition, low 25(OH)D levels may contribute to pro-atherosclerotic changes of vascular smooth muscle cells, endothelial dysfunction and increased macrophage to foam cell formation.
- High dose oral vitamin D supplementation produced short-term

improvement in endothelial function in stroke patients with wellcontrolled baseline blood pressure.

- Finally, low 25(OH)D levels are known to influence macrophage and lymphocyte activity in atherosclerotic plaques and to promote chronic inflammation in the artery wall. Various studies suggest that vitamin D may exert anti-inflammatory effects. Reduced 25(OH)D levels might be associated with overall increased inflammatory activity.

In a study conducted by **Kenneth et al**[49] in 2005, 77% of patients with acute ischaemic stroke had Vitamin D insufficiency.This was one of the pioneer studies involving Vitamin D conducted in United States of America. They evaluated the levels of 25 hydroxy Vitamin D at the onset of stroke and 30 days later and found significant Vitamin D deficiency at the onset of stroke and during followup studies.

In a study conducted by **Stefan Pilz et al**[50] from 1997-2000, it was found that lower Vitamin D levels have independent predictive value in fatal strokes and Vitamin D supplementation can prevent fatal strokes. They found 58% prevalence of Vitamin D deficiency in stroke patients.

In a recent study conducted by **Tu WJ et al in China**[51] from 2010 to 2012, it was found that the mean 25 hydroxy Vitamin D levels in patients with acute ischaemic stroke was 10.2–18.9ng/ml and in normal controls it was 17.5-22.9ng/ml. They also concluded that Vitamin D levels is an independent

predictor of mortality after acute ischaemic stroke within 90 days of stroke episode.

In 2013, **the Vitamin D council,**[52] in its statement declared that Vitamin D deficiency is an important global problem with significant association to stroke. It suggested many groups to do further studies into the association between Vitamin D and stroke and the pathogenesis behind it.

The **Ludwigshafen Risk and Cardiovascular Health (LURIC) Study**[53] conducted from 1997 to 2000 found that over a period of 7.7 yrs of followup after acute stroke, 92% patients had below normal Vitamin D levels. But whether this was the cause for stroke or the after effect of stroke could not be predicted from this study. But they suggested a definite link between acute ischaemic stroke and Vitamin D.

NHANES study(National Health and Nutrition Examination Survey)[54] showed that over a median of 14years, whites with low vitamin D levels had double risk of stroke compared to those having higher levels of vitamin D.

A study done by **Deidre A de Silva et al**[55], showed that in Asian population, 95% of acute ischaemic stroke patients had below normal Vitamin D compared to 84% in controls with 39% of cases and 20% of controls having Vitamin D deficiency.

VITAMIN D DEFICIENCY AND BONE HEALTH

Vitamin D plays a pivotal role in maintaining serum calcium and phosphorous. Without vitamin D, only 10-15% of dietary calcium and 60% of phosphorous is absorbed.[12-14] Thus, Vitamin D is an integral part of skeletal mineralization.

Vitamin D deficiency causes secondary hypoparathyroidism which leads to osteopenia and osteoporosis by increasing bone resorption. As a result of raised parathormone, phosphaturia and hypophosphataemia occur causing defective mineralization of bone osteoid.

Rickets and osteomalacia are widely prevalent in India[29]. Low peak bone mass leads to pseudofractures. On routine screening, there is wide prevalence of biochemical osteoporosis and osteomalacia in our population. The benefits of 25hydroxy vitamin D on skeletal health starts from early fetal life and continues upto adulthood.[12]

Hollick et al, Dawson Hughes et al and many others [26] have linked low levels of 25 hydroxy vitamin D to fractures. Osteoarthritis of hip and knee joint has also been associated with vitamin D deficiency.

Surveys from rural south India (Tirupati) have shown that Vitamin D levels are higher in agricultural workers who are exposed to long hours of sunlight as compared to urban dwellers (24ng/ml vs 19ng/ml)[39-41]. Serum

Vitamin D levels were significantly lower than expected for the duration of sunlight exposure, inspite of high exposure to sunlight. Studies on dietary habits of this population have shown that these persons habitually consume low-calcium, high-phytate diets. Of the daily diet of 1700 KJ/day approximately in these rural individuals, carbohydrates provided 75% of the total energy intake, fat 5%,proteins 10%, vegetables 5%, and milk and milk products 5%. The carbohydrate sources were cereals [Rice – 60% and Ragi-40%]. Animal sources of protein were consumed approximately once in 2 weeks only.

In the diets of urban individuals, with a total energy intake of 2200 KJ/day approximately, carbohydrates provided 55% of the total energy intake, proteins 10%, fat 10%, vegetables 10%, and milk and milk products 15%. The carbohydrate sources were primarily cereals (rice 50%, wheat 25%, and ragi 25%). Animal sources of protein were consumed only once a week. There was no other source of calcium or any other mineral in either of the groups. Milk in India is not fortified with calcium or vitamin D.

The daily dietary calcium intake reported in both rural and urban populations in the Tirupati study were low (mean + SEM: rural 264 ± 1.94; urban 354 ± 5 mg/day) as compared to the Recommended Daily/Dietary Allowance (RDA) for Indians(800mg/day). The consumption of Ragi (rich in phytates) by the rural population retards the absorption of calcium from the gut. Similar calcium-deficient diets have been reported in other Indian studies as

well . The average dietary calcium intake in India seems to be 430 ± 180 mg/day in children and 560 ± 310 mg/day in adults . All studies have uniformly documented low dietary calcium intake as compared to the ICMR's RDA norms.

Low calcium intake increases parathyroid hormone (PTH), which in turn increases conversion of 25(OH)D to 1,25-dihydroxyvitamin D. In addition, 1,25-dihydroxyvitamin D induces its own destruction by increasing 24-hydroxylase . This probably explains the low 25hydroxy vitamin D concentrations in persons on a high-phytate or a low-calcium diet. It is, therefore, essential that calcium supplementation should be made an integral part of vitamin D supplementation therapy in India.

VITAMIN D DEFICIENCY AND TYPE 2 DIABETES MELLITUS

The current prevalence of Type 2 Diabetes mellitus is high both in urban and rural India.[56] By the year 2030, it is estimated that India would have the maximum number of diabetics in the world.[57]

A study done by **Pittas et al**[58] has shown that increased risk of type 2 Diabetes mellitus when serum 25 hydroxy Vitamin D levels fall below 30 ng/ml. It has also been proven that glycemic status worsens in Winter which is associated with reduced Vitamin D levels.

Vitamin D receptors are present in the pancreas and it also has 1α hydroxylase activity. Hence it can convert 25 hydroxy Vitamin D into 1,25 dihydroxy Vitamin D in a minor way and act in a paracrine or autocrine fashion.

In a recent study, one group has shown that optimal treatment with vitamin D as per current Endocrine Society guidelines and supplementation with calcium improves pancreatic beta cell function in normoglycaemic subjects with vitamin D deficiency.

Mechanisms by which Vitamin D prevents Diabetes mellitus:

1. Enhances insulin release by improving beta cell function, either directly or by increasing the intracellular ionised calcium level.
2. Inhibits beta cell apoptosis[57]
3. Increases the sensitivity of calcium dependent pathways in target cells that enhance glucose utilisation
4. Increases the expression of insulin receptors, thus increasing insulin sensitivity.

VITAMIN D DEFICIENCY AND SYSTEMIC HYPERTENSION

Pfeifer et al[59] showed a 9% fall in systolic blood pressure after supplementing 800 IU of Vitamin D. In another study, in patients exposed to UVB rays for 3 months thrice a week, there was 180% increase in Vitamin D levels and 6 mmHg reduction in both systolic and diastolic BP.

On the contrary, **Forman et al**[60] showed no correlation between hypertension and Vitamin D supplementation.

Mechanisms implicated are:

- Direct effect on endothelial cells
- Regulation of calcium metabolism
- Suppression of Renin Angiotensin Aldosterone axis
- Norepinephrine and Angiotensin II play a main role in the pathogenesis of hypertension. Vitamin D has a role in the regulation of these.

VITAMIN D IN CHRONIC RENAL FAILURE

Patients with chronic kidney disease treated with Vitamin D have shown a fall in death rates by 20%.[49] Antiproteinuric effect of Vitamin D has also been demonstrated.[49] A study done by **Williams et al**[61] found that patients with chronic renal failure had severe Vitamin D deficiency and Vitamin D supplementation over a 3 month period had significant reduction in morbidity in these patients. Low vitamin D also leads to high incidence of cardiovascular events in chronic renal failure patients.

VITAMIN D AND ATHEROSCLEROSIS

- Parathormone promotes the formation of intra-arterial plaque, thus

increasing the risk of atherosclerosis. Hence calcification and stenosis in the blood vessels is reduced by suppressing parathormone activity.

- Vitamin D maintains normal vascular tone by promoting nitric oxide

production, suppressing platelet aggregation and thrombogenic activity. Vitamin D governs many bone proteins like matrix G1a protein and osteoprotegerin which are present in the blood vessel wall. In case of vitamin D deficiency, these proteins cause calcification of vessels.

- Vitamin D has anti-inflammatory activity. It affects macrophages and

dendritic cells, reducing foamy macrophages and suppressing cholestrol uptake.

- Moreover, few cardiac drugs increase Vitamin D levels. Vitamin D levels

are increased by 70% after 1 year of treatment with statins. Drugs like beta blockers, aspirin, thiazide diuretics,etc. have shown to enhance Vitamin D activity.

- Vitamin D reduces risk of diabetes mellitus and hypertension, thereby

reduces the risk of atherosclerosis.

VITAMIN D AND CORONARY ARTERY DISEASE

In a large randomized trial by **Wang et al[62],** the relative risk of developing myocardial infarction was 3 times more in individuals with Vitamin D deficiency when compared with individuals with normal Vitamin D levels. Vitamin D acts mainly by gene transcription and maintaining calcium homeostasis to prevent cardiovascular diseases and its risk factors.

Mechanisms for cardiovascular protective effect include-

- Protective action on the endothelium
- Inhibition of renin
- Regulation of parathormone
- Anti- inflammatory action
- Plaque stability
- Preventing cardiac hypertrophy
- Reduced cardiac contractility
- Reduced risk of arrythmias

VITAMIN D AND DYSLIPIDEMIA

A study done by **Chaudhuri et al**[71] in 2013 in India showed that people with Vitamin D deficiency have dyslipidemia, as shown by increased LDL cholestrol, decreased HDL cholestrol,increased total cholestrol and increased triglycerides. Significant association was shown when the vitamin D levels were less than 20ng/ml.

Another study done by **Zittermann et al**[72] in Europe also found a significant association between Vitamin D and dyslipidemia. The mechanisms are mostly speculative with none being proved scientifically.

MUSCLE STRENGTH AND VITAMIN D

Muscle strength plays an important role in determining risk for falls, which result in fractures and other injuries. Muscle wasting is a multifactorial process involving intrinsic and extrinsic alterations. There are studies to show moderate inverse relationship between vitamin D status and muscle strength .

Randomized controlled trials (RCTs) of the effect of vitamin D/calcium supplementation on skeletal muscle strength have not shown positive effects in the elderly. Oral cholecalciferol/calcium supplementation in the dose/schedule that is generally used for increasing and maintaining serum 25(OH)D did not lead to improved skeletal muscle strength in young women.

SKIN DISEASES AND VITAMIN D

Psoriasis is a semi-autoimmune disease which affects approximately 50 million people worldwide. It affects mostly adults and is characterized by raised patches of thick, red skin covered with silvery scales. These patches are sometimes called plaques, which generally itch and may burn. Under normal circumstances, skin cells grow, divide and replace themselves in an orderly fashion. But in psoriasis, cells start reproducing in an uncontrolled manner.

Psoriatic skin may “turn over” (be replaced) in as little as four days as compared to normal skin which turns over in twenty-one days. Local application of skin ointment of activated vitamin D (calcitriol) dramatically reduces the symptoms of psoriasis.

VITAMIN D DEFICIENCY AND AUTOIMMUNITY

Vitamin D deficiency has been linked to many autoimmune diseases like Type 1 diabetes mellitus, multiple sclerosis, rheumatoid arthritis and inflammatory bowel disease. This is attributed to the fact that Vitamin D receptors are present on monocytes, dendritic cells, macrophages, WBCs, CD4 and CD8 T cells. Thus, the immune system of the body is also affected by Vitamin D. Cytokine production and T cell proliferation are inhibited by Vitamin D.[63]

Epidemiological data show correlation between the seasonal variation in the onset of these autoimmune diseases with vitamin D deficiency.[63] Vitamin D improves and prevents Multiple sclerosis by increasing TGF β levels.[49]

VITAMIN D DEFICIENCY AND TUBERCULOSIS

Before the advent of anti tubercular drugs, high doses of Vitamin D and cod liver oil were used to treat tuberculosis in the 18th and 19th century. This was done on the basis that Vitamin D would calcify the tuberculous lesions.[64] Vitamin D deficiency is an independent risk factor for Tuberculosis in South Asians.[64] This is mainly because Vitamin D promotes killing of the intracellular mycobacteria by increasing cathelicidin in the macrophages.

Patients with chronic granulomatous diseases such as sarcoidosis, and those with tuberculosis or fungal infections are at risk of vitamin D deficiency, because their immune systems are activating the vitamin D. They need to be treated for vitamin D deficiency but should receive much smaller doses of vitamin D than patients who are otherwise normal and are being treated for vitamin D deficiency alone. Otherwise they may develop hypercalcemia and hypercalcuria. The 25(OH)D levels in such patients should be maintained between 20-30 ng/ml.

VITAMIN D DEFICIENCY AND MALIGNANCY

Certain malignancies like Hodgkins Lymphoma, colon, prostrate, ovarian, pancreatic, breast carcinoma and few others are more prevalent among people living in higher latitudes. Low Vitamin D in these regions is attributed as one of the reasons. Vitamin D induces apoptosis, regulates cell cycle and cell differentiation. It inhibits tumor growth and metastasis. **30-50% reduction** in the risk of malignancy after Vitamin D supplementation as been shown by few studies.[65]

Another study showed the beneficial effect of sunlight on both breast cancer and prostate cancer . Cancers of the digestive tract (colon, rectum, mouth, esophagus, stomach and pancreas) are also associated with low 25(OH)D levels .

Ethnicity may also have a role to play. Vitamin D deficiency was found to be more prevalent and pronounced in African Americans than in Caucasian Americans . It has been reported that, after adjusting for multiple dietary, lifestyle and medical risk factors, African American men were at 32% greater risk of total cancers and especially cancers of digestive tract than their Caucasian counterparts.

About 75% of women with breast cancer who are vitamin D deficient at diagnosis die from the disease while mortality risk is lower in women with normal vitamin D levels at diagnosis.

Data analysis from the **National Health and Nutrition Examination Survey [NHANES I]**[67] in 1999 demonstrated that increased exposure to sunlight could, by itself, potentially reduce the incidence and death rate of breast cancer in the Unites States by 35 to 75% .

Results pooled from the **Harvard Nurses Health study and St. Georges Hospital study in London**[66] showed that patients with high 25 hydroxy Vitamin D levels had the lowest risk of breast cancer .

Prostate cancer is fatal in about 25% of the cases. It has been reported that the risk of developing prostate cancer is inversely related to the level of exposure to sunlight . Men with prostate cancer who received 2000 IU of vitamin D daily were shown to have a 50% reduction in risk as measured in terms of the levels of prostatic specific antigen (PSA), an indicator of cancer activity. Those living at higher altitudes are generally at increased risk of developing cancer[66].

Studies from Creigton University[67] reported that postmenopausal women who took 1500 mg/day of calcium and 1100 IU/day of vitamin D for four years had a 60% reduction in the risk of developing all cancers as compared to placebo group .

ROLE OF VITAMIN D IN OTHER DISEASES

Crohn's disease affects the proximal small intestine and hampers 25(OH)D absorption. Recent advances in understanding the pathophysiology of Crohn's disease have revealed the so-called north– south gradient of Crohn's disease . In a genetically predisposed individual, Crohn's disease occurs because of the dysregulated response of the mucosal immune system to intraluminal antigens of bacterial origin. A normally functioning mucosal immune system inhibits immune response to luminal antigens and suppresses gut inflammation (immune tolerance).

The mechanism whereby exposure to sunlight is thought to exert a beneficial effect on intestinal inflammation may involve vitamin D. Sunlight and vitamin D might protect against Crohn's disease by down-regulating the T helper-1 (TH1)-driven immune response. The mechanism through which heliotherapy (UV-B rays) induces immune suppression may include the induction of various TH 2 cytokines such as IL-4 and IL-1012.

Vitamin D may be the coordinator of the cross talk between the immunological system in the gut and various subcellular events in bone formation. Approximately 10% of the population has silent Coeliac disease. These individuals have difficulty in absorbing fat-soluble vitamin D. Unless they have enough UV-B to maintain healthy vitamin D levels, they should receive vitamin D supplementation to maintain their 25(OH)D levels at >30 ng/ml.

Cystic fibrosis leads to malabsorption of vitamin D. Patients with this disease require aggressive supplementation with vitamin D to maintain 25(OH)D levels at > 30ng/ml.

In **cirrhosis of the liver** when more than 80% of the liver is destroyed, there is decreased production of 25(OH)D and poor absorption of fat as well as of vitamin D. Mild to moderate malabsorption is a major cause of vitamin D deficiency in these patients. A similar situation prevails in primary biliary cirrhosis. These conditions call for aggressive treatment with vitamin D.

DIAGNOSIS OF VITAMIN D DEFICIENCY

The most sensitive marker to assess the vitamin D status in the general population is 25 hydroxy Vitamin D, which is the main circulating form of Vitamin D. This is because it has a longer half life of around 2-3 weeks and can be easily measured. Moreover, clinical disease states correlate well with serum 25 hydroxy Vitamin D levels.

1,25dihydroxy Vitamin D has a shorter half life of 15hrs and is easily affected by calcium, phosphorous and parathormone levels, hence it is a poor indicator of Vitamin D deficiency. Also, its levels fall only when there is severe Vitamin D deficiency. Usually serum calcium is found to be normal in people with Vitamin D deficiency due to effective compensatory mechanisms like increased parathormone levels.

METHODS TO ASSESS 25 HYDROXY VITAMIN D LEVELS

- **Ligand Binding Assays**
  - Radioimmunoassay
  - Chemiluminescence assay
  - Competitive protein-binding assays
- **Liquid chromatography – tandem mass spectrometry (LC-MS/MS)**
  - Accurate and precise
  - Considered the 'Gold Standard method'
- **High performance liquid chromatography (HPLC)**

TREATMENT OF VITAMIN D DEFICIENCY

Dosing[68]

As per the Institute of Medicine (IOM), the level of deficiency and treatment with Vitamin D supplementation should be individualised.

- 25hydroxy Vitamin D < 20ng/ml (50nmol/L) – 50,000 IU of Vitamin D2 or D3 orally once a week for 6-8weeks, followed by 800-1000 IU of Vitamin D3 daily.
- 25hydroxy Vitamin D 20- 30 ng/ml (50 - 75 nmol/L) – 800 to 1000 IU of Vitamin D3 daily for 3 months
- In infants and children with 25hydroxy Vitamin D < 20ng/ml (50 nmol/L) – 1000 to 5000 IU of Vitamin D2 daily for 2-3 months.

- 1200 mg of calcium daily for postmenopausal women and 1000 mg daily for prmenopausal women should be supplemented along with Vitamin D.

MONITORING BLOOD LEVELS

Blood levels of 25 hydroxy Vitamin D should be monitored every 3 months after starting treatment and dose adjustments made accordingly.

PREVENTION OF VITAMIN D DEFICIENCY

Vitamin D deficiency and its complications can be prevented by adequate sun exposure, fortification of foods, public awareness campaigns and adequate Vitamin D supplementation in vulnerable population. Vitamin D supplementation must be properly advised to the people and the dosing be adjusted according to degree of sun exposure, skin colour, diet and underlying medical conditions.

Recommended Dietary Allowances (RDAs) for Vitamin D[68]

Age	Male	Female	Pregnancy	Lactation
0-12 months	400 IU	400 IU		
1-13 yrs	600 IU	600 IU		
14-18 yrs	600 IU	600 IU	600 IU	600 IU
19-50 yrs	600 IU	600 IU	600 IU	600 IU
51-70 yrs	600 IU	600 IU		
>70 yrs	800 IU	800 IU		

(40 IU = 1mcg)

The current recommendation is to take 2000IU of Vitamin D and 1-1.5 gm of calcium daily in order to prevent Vitamin D deficiency in the Indian population.

In March 2007, a group of Vitamin D researchers published a controversial editorial mentioning the desirable concentration of 25 hydroxy Vitamin D was ≥ 30 ng/ml (≥75nmol/L).[69] They told that approximately 1700 IU/day of Vitamin D is needed to raise 25 hydroxy Vitamin D levels from 20ng/ml to 32ng/ml. However, the FNB committee that recommended the Recommended Daily Allowance for Vitamin D extensively reviewed a wide range of articles and came to the conclusion that with the exception of measures related to bone health, the health relationships examined were either not

supported by satisfactory evidence or had a conflicting nature of evidence, hence specific levels of Vitamin D intake cannot be linked to any of these conditions.

VITAMIN D – DRUG INTERACTIONS

Drugs that decrease the serum levels of Vitamin D by increasing its metabolism

Phenytoin, Fosphenytoin, Phenobarbitone, Carbamazepine, Non-nucleoside reverse transcriptase inhibitors, Rifampin, Theophylline, Cimetidine

Decrease the intestinal absorption of Vitamin D

Cholestyramine, Colestipol, Mineral oil, Orlistat

Others:

Ketoconazole – reduces serum levels of Vitamin D by inhibiting 1α hydroxylase enzyme

Corticosteroids – impair Vitamin D metabolism and calcium absorption

Thiazide diuretics and statins – increase serum Vitamin D levels

Vitamin D toxicity – can precipitate cardiac arrythmias by causing hypercalcemia, especially in patients on digoxin

HYPERVITAMINOSIS D

It is extremely rare. It can result from poisoning or when Vitamin D deficiency is treated without monitoring serum levels.

Doses more than 10,000 IU/day can cause serum levels of 25 hydroxy Vitamin D to go above 150 ng/ml causing acute hypercalcemia and hyperphosphatemia. Lymphoma and primary hyperparathyroidism also can increase the risk of hypercalcemia in response to Vitamin D.[70]

TOLERABLE UPPER INTAKE LEVEL FOR VITAMIN D[70]

As per the Institute of Medicine (IOM),

Infants: 0-6 months – 1000 IU/day

6-12 months – 1500 IU/day

Children: 1-3 yrs – 2500 IU/day

4-8yrs – 3000 IU/day

Adults: > 9yrs – 4000IU/day

As per the European Food and Safety Authority (EFSA),

0-10yrs – 1000 IU/day

>11yrs – 2000 IU/day

CLINICAL FEATURES OF HYPERVITAMINOSIS D

- headache, dehydration, lethargy
- constipation, loss of appetite
- nausea, vomiting, abdominal pain
- failure to thrive (in children)
- renal stones
- polyuria, polydipsia
- increased 1α hydroxylase activity led to premature ageing in mice
- increased risk of vascular calcification and pancreatic cancer has been associated with serum levels of 25 hydroxy vitamin D > 60 ng/ml.

MANAGEMENT OF HYPERVITAMINOSIS D

It needs treatment with intravenous fluids, steroids and calcium restricted diet. Bisphosphonates may be needed to control hypercalcemia.

The wide spectrum of action of Vitamin D continues to intrigue the scientific community. The calcemic beneficial effects of vitamin D have been fully established through outcome studies, and the guidelines for treatment of vitamin D deficiency for calcemic benefit are established. The non-calcemic beneficial effects are gradually becoming better understood. While we await guidelines for vitamin D supplementation for non-calcemic benefits, it may be prudent to maintain the serum 25(OH)D levels at 30 ng/ml, and also ensure a diet-cum-supplement calcium intake of 1 gm per day.

MATERIALS

AND

METHODOLOGY

MATERIALS AND METHODOLOGY

PLACE OF STUDY: Government Kilpauk Medical College & Hospital, Chennai-600010

STUDY DESIGN: Observational Case- Control Study

DURATION OF THE STUDY: 6 months

PERIOD OF STUDY: March 2014 to August 2014

SAMPLE SIZE: 50 cases and 50 controls (age and sex matched)

COLLABORATING DEPARTMENTS:

Department of Neurology, Department of Biochemistry, Department of Radiology, Government Kilpauk Medical College & Hospital.

CONFLICT OF INTEREST:

There was no conflict of interest.

ETHICAL CLEARANCE:

Obtained. The study protocol was approved by the Ethical Committee of Government Kilpauk Medical College & Hospital,Chennai-10 for research studies conducted in February, 2014.

INFORMED CONSENT:

Both the case and control study groups were informed about the nature of the study. Members who were willing to participate in this study were included after getting their written informed consent.

Patients who fulfil the inclusion and exclusion criteria were enrolled in the study.

INCLUSION CRITERIA:

1. Patients with new onset stroke with an acute infarct on CT Brain, admitted in Medical Wards in Governemnt Kilpauk Medical College Hospital, within 7days of onset of stroke were taken as cases.
2. Patients without stroke, who attended the Medical OPD were taken as Controls (Age and sex matched).

EXCLUSION CRITERIA:

Patients with -

1. History of Transient Ischaemic Attacks, prior stroke
2. Diabetes mellitus
3. Systemic hypertension
4. Coronary artery disease

5. Chronic kidney disease
6. On drugs that affect Vitamin D metabolism

   (Anti epileptics, Steroids, Rifampin)
7. Calcium or vitamin D supplements

DATA COLLECTION:

A data collection form was prepared to note the Name, Age, Sex, Smoking, Drug Intake and other relevant history, Height and Weight of the cases and controls.

The CT Brain reports of the cases were noted.

LABORATORY INVESTIGATIONS:

- Blood samples were taken at the time of admission to measure the serum levels of 25 hydroxy Vitamin D level and lipid profile (HDL cholestrol, LDL cholestrol, Total Cholestrol and triglycerides).
- Serum levels of 25 hydroxy Vitamin D levels were measured by Chemi Luminescence Immuno Assay Technique.
- Lipid profile was measured using enzymatic methods.
- Tests were done in a single laboratory by the same person. Therefore no interpersonal error was possible.

NORMAL VALUE OF THE PARAMETERS:

The normal value of the parameters assessed is listed below.

- Vitamin D levels

  Normal 30-100ng/ml

  Insufficient 10-30ng/ml

  Deficient <10ng/ml

- Obesity- based on Body Mass Index (for Indian population)

  BMI = weight(kg)/Height^2(m^2)

  Normal- 18.0-22.9 kg/m^2

  Overweight- 23.0-24.9 kg/m^2

  Obese >25 kg/m^2

- Lipid Profile - normal values (NCEP guidelines)

  Serum triglycerides <150mg/dl

  LDL Cholesterol <100mg/dl

  HDL cholesterol >40 mg/dl for men

  >50 mg/dl for women

  Total cholesterol <200mg/dl

Statistical Tools

- The data collected regarding all the selected cases was recorded in a Master Chart. Data analysis was done with the help of computer using standard SPSS software package (Statistics Products Services Solutions).
- Using this software range, frequencies, percentages, means, standard deviations, ‘T’ test, chi square and 'p' values were calculated.
- Kruskul Wallis chi-square test was used to test the significance of difference between quantitative variables and Yate’s test for qualitative variables.
- 'p' value < 0.05 is taken to denote significant relationship.
- ‘p’ value <0.01 is taken to denote highly significant relationship.

RESULTS

OBSERVATIONS AND ANALYSIS

STUDY POPULATION CHARACTERISTICS

This study included a total of 100 subjects out of which 50 were cases (acute ischaemic stroke) and 50 were controls.

TABLE 1:

	Frequency	Percent	Valid Percent	Cumulative Percent
Case	50	50	50	50
Control	50	50	50	50
Total	100	100	100	100

FIGURE 1:

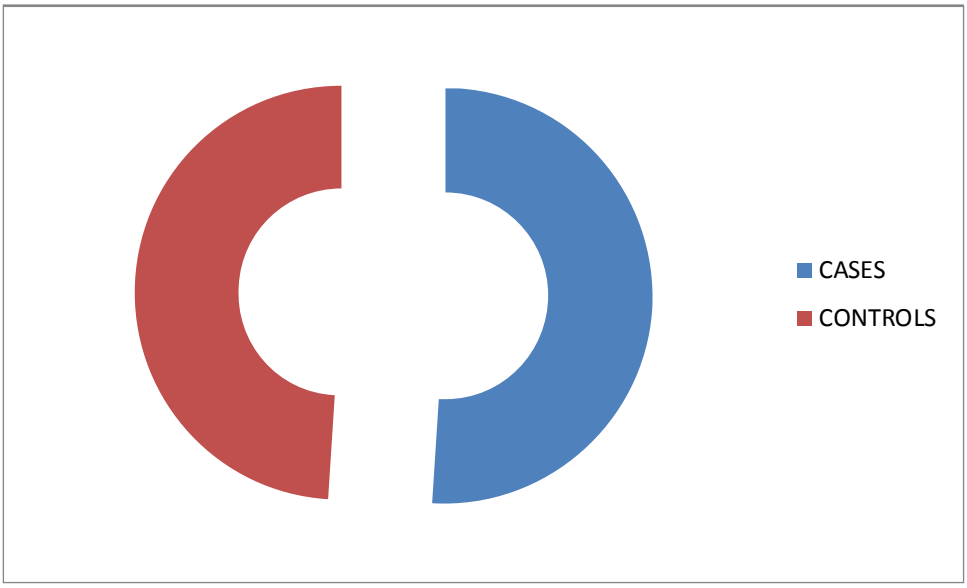
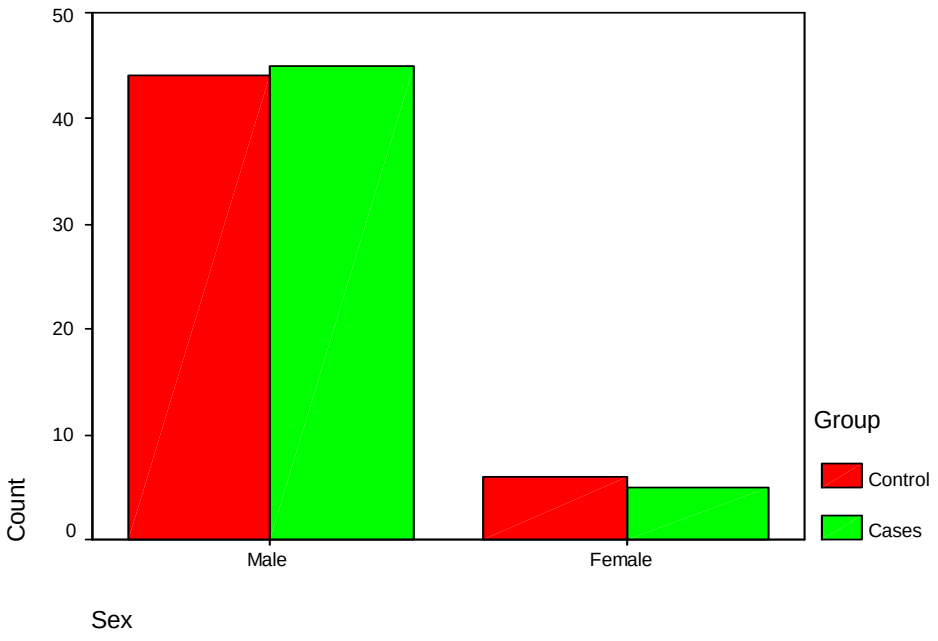


TABLE 2: GENDER WISE DISTRIBUTION OF PATIENTS

			Group		Total
			Control	Cases	
Sex	Male	Count	44	45	89
		% within Sex	49.4%	50.6%	100.0%
		% within Group	88.0%	90.0%	89.0%
	Female	Count	6	5	11
		% within Sex	54.5%	45.5%	100.0%
		% within Group	12.0%	10.0%	11.0%
Total		Count	50	50	100
		% within Sex	50.0%	50.0%	100.0%
		% within Group	100.0%	100.0%	100.0%

p value – 0.749 (not significant)

FIGURE 2: GENDER WISE DISTRIBUTION OF PATIENTS



The 50 cases studied included 45 (90%) males and 5 (10%) females. The 50 controls studied included 44 (88%) males and 6 (12%) females.

There was not statistically significant difference.

Inference:

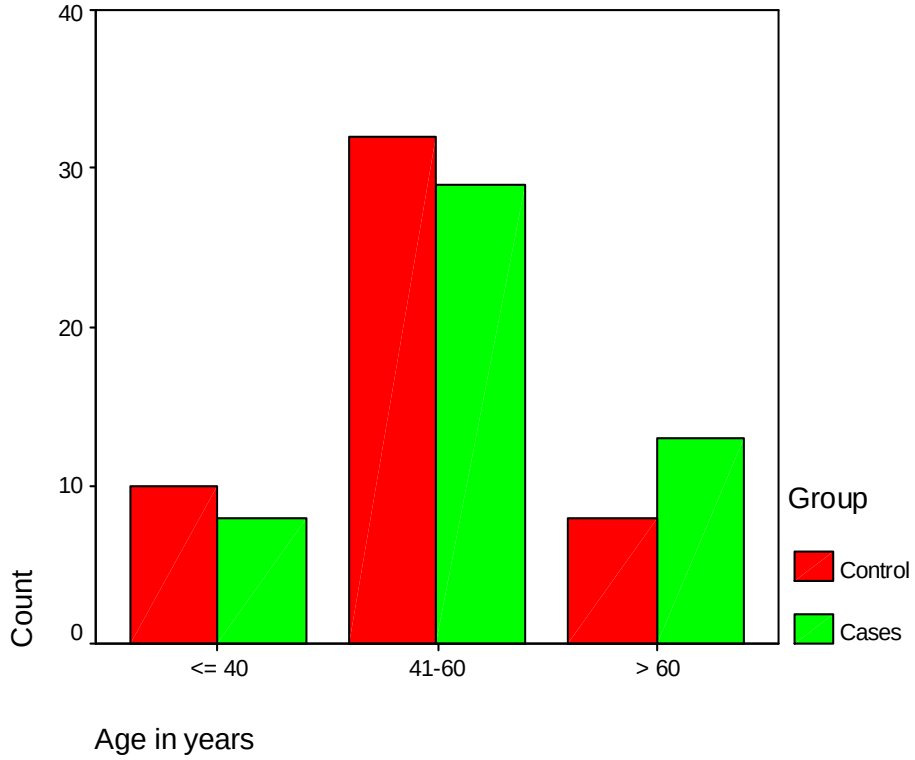
The sex composition of the cases and controls did not differ significantly.

TABLE 3: AGE WISE DISTRIBUTION OF PATIENTS

			Group		
			Control	Cases	Total
Age in years	<= 40	Count	10	8	18
		% within Age in years	55.6%	44.4%	100.0%
		% within Group	20.0%	16.0%	18.0%
	41-60	Count	32	29	61
		% within Age in years	52.5%	47.5%	100.0%
		% within Group	64.0%	58.0%	61.0%
	> 60	Count	8	13	21
		% within Age in years	38.1%	61.9%	100.0%
		% within Group	16.0%	26.0%	21.0%
Total		Count	50	50	100
		% within Age in years	50.0%	50.0%	100.0%
		% within Group	100.0%	100.0%	100.0%

p value – 0.458 (not significant)

FIGURE 3 : AGE WISE DISTRIBUTION OF PATIENTS



The study population was stratified into 3 groups according to their age. The mean age in the case and control groups were 51.98 ± 13.114 and 51.80 ± 10.633 years respectively.

Inference:

No significant difference in the age distribution between the cases and controls in all the age groups.

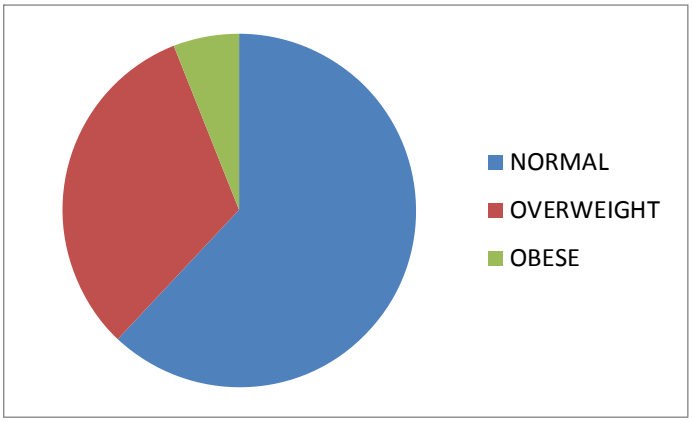
TABLE 4: CASES AND CONTROLS WITH RESPECT TO BMI

			BODY MASS INDEX(kg/m^2)			Total
			Normal	Overweight	Obese	
GROUP	Case	Count	19	17	14	50
		% within group	38%	34%	28%	100%
	Control	Count	30	17	3	50
		% within group	60%	34%	6%	100%
Total		Count	50	32	18	100
		% within group	50%	32%	18%	100%

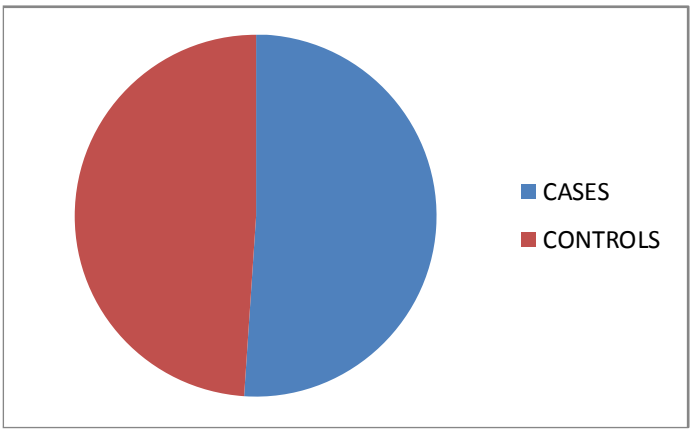
P value – 0.001 (significant)

FIGURE 4 : CASES AND CONTROLS WITH RESPECT TO BMI

CASES



CONTROLS



	Group	N	Mean	Std. Deviation	Std. Error Mean
BMI	Control	50	**24.5016**	2.83610	.40109
	Cases	50	**26.8170**	3.71224	.52499

Inference:

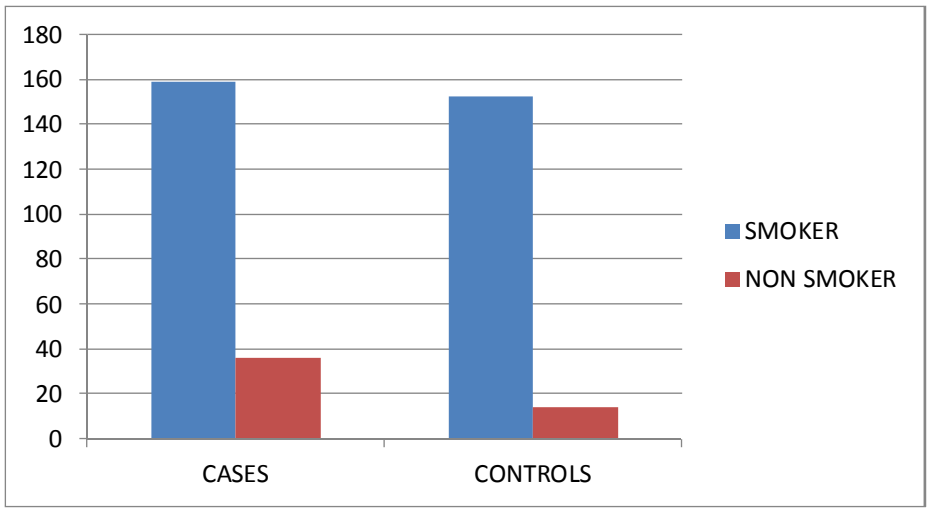
The BMI of the study group is significantly higher than that of the control group.

TABLE 5 : CASES AND CONTROLS WITH RESPECT TO SMOKING

			Group		Total
			Control	Cases	
Smoking	Yes	Count	36	38	74
		% within Smoking	48.6%	51.4%	100.0%
		% within Group	72.0%	76.0%	74.0%
	No	Count	14	12	26
		% within Smoking	53.8%	46.2%	100.0%
		% within Group	28.0%	24.0%	26.0%
Total		Count	50	50	100
		% within Smoking	50.0%	50.0%	100.0%
		% within Group	100.0%	100.0%	100.0%

p value – 0.648 (not significant)

FIGURE 5 : CASES AND CONTROLS WITH RESPECT TO SMOKING



There is no significant difference between the cases and controls with reference to smoking.

Inference:

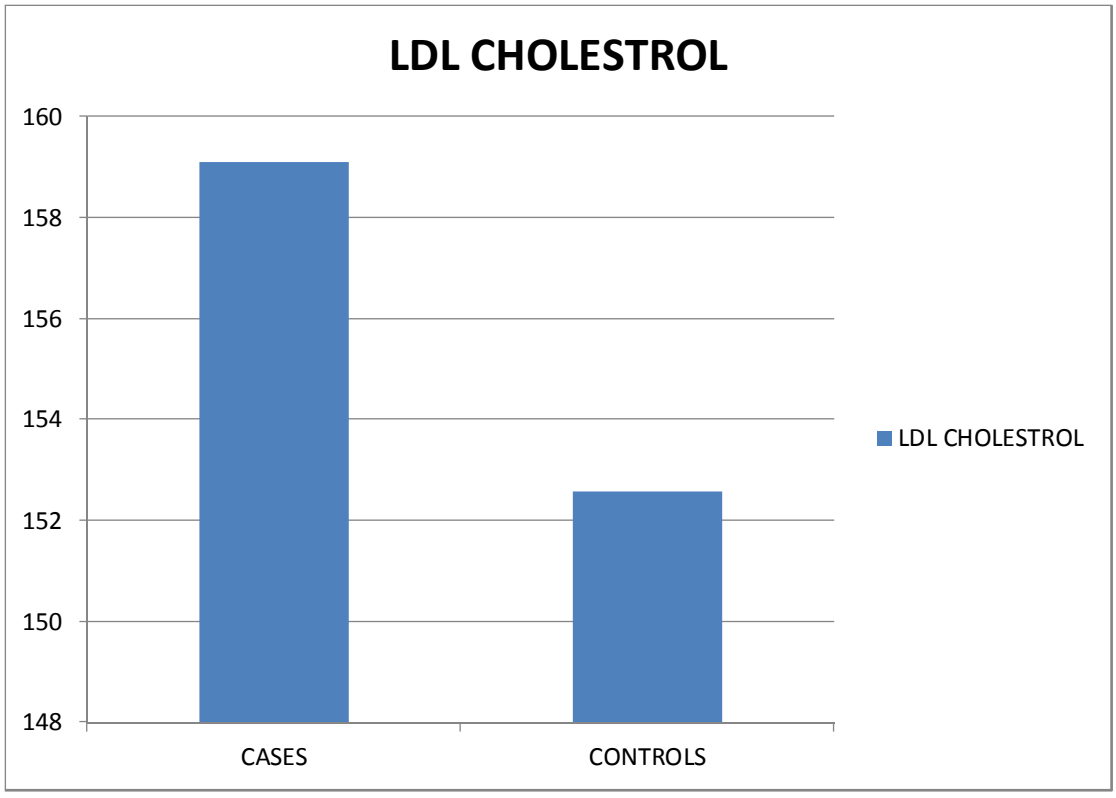
We could safely exclude smoking as a confounding factor for cerebrovascular risk.

TABLE 6 : CASES AND CONTROLS WITH RESPECT TO LDL CHOLESTROL

LDL	Group	No.	Mean	Std. Deviation	Std. Error Mean
	Cases	50	**133.26**	32.855	4.646
	Control	50	**118.20**	29.596	4.185

p value – 0.18 (not significant)

FIGURE 6 : CASES AND CONTROLS WITH RESPECT TO LDL CHOLESTROL



The mean LDL cholestrol among cases and controls is 133.26 and 118.20 mg/dl and a standard deviation of 32.855 and 29.596 respectively, p value – 0.18.

Inference:

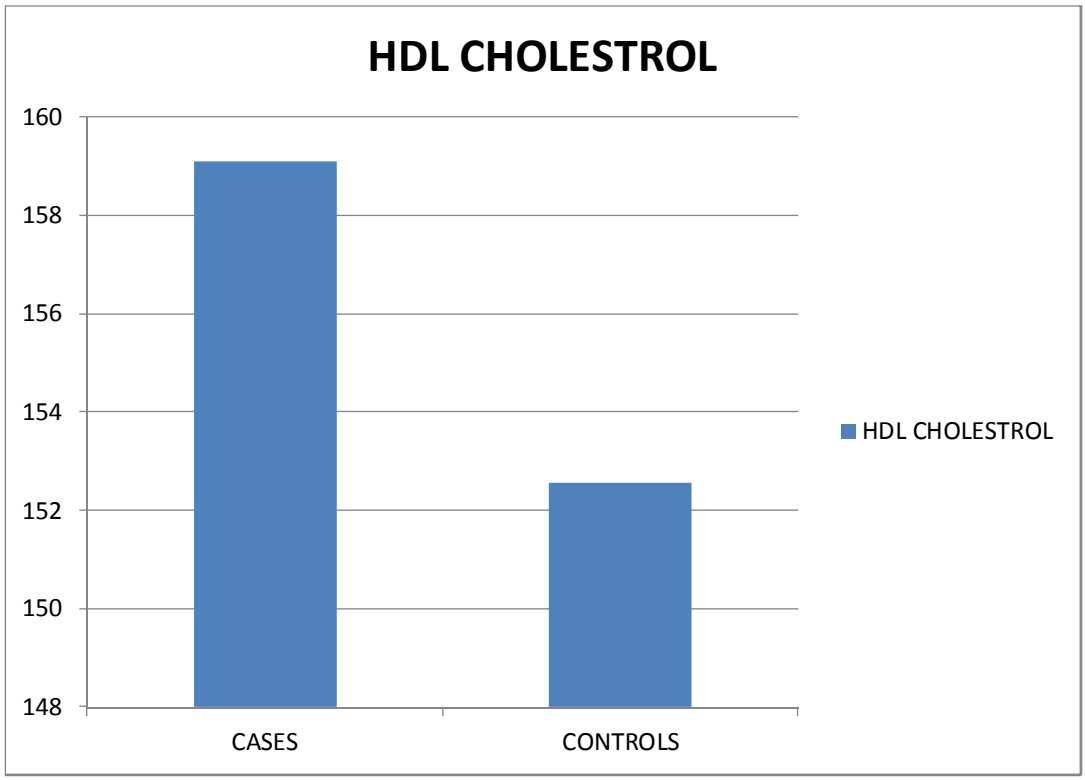
The difference between the two groups with respect to LDL cholestrol is not statistically significant.

TABLE 7 : CASES AND CONTROLS WITH RESPECT TO HDL CHOLESTROL

HDL	Group	No.	Mean	Std. Deviation	Std. Error Mean
	Cases	50	**44.24**	5.850	.827
	Control	50	**43.86**	6.704	.948

p value – 0.763 (not significant)

FIGURE 7 : CASES AND CONTROLS WITH RESPECT TO HDL CHOLESTROL



The mean HDL cholestrol among cases and controls is 44.24 and 43.86 mg/dl and a standard deviation of 5.85 and 6.704 respectively, p value – 0.763

Inference:

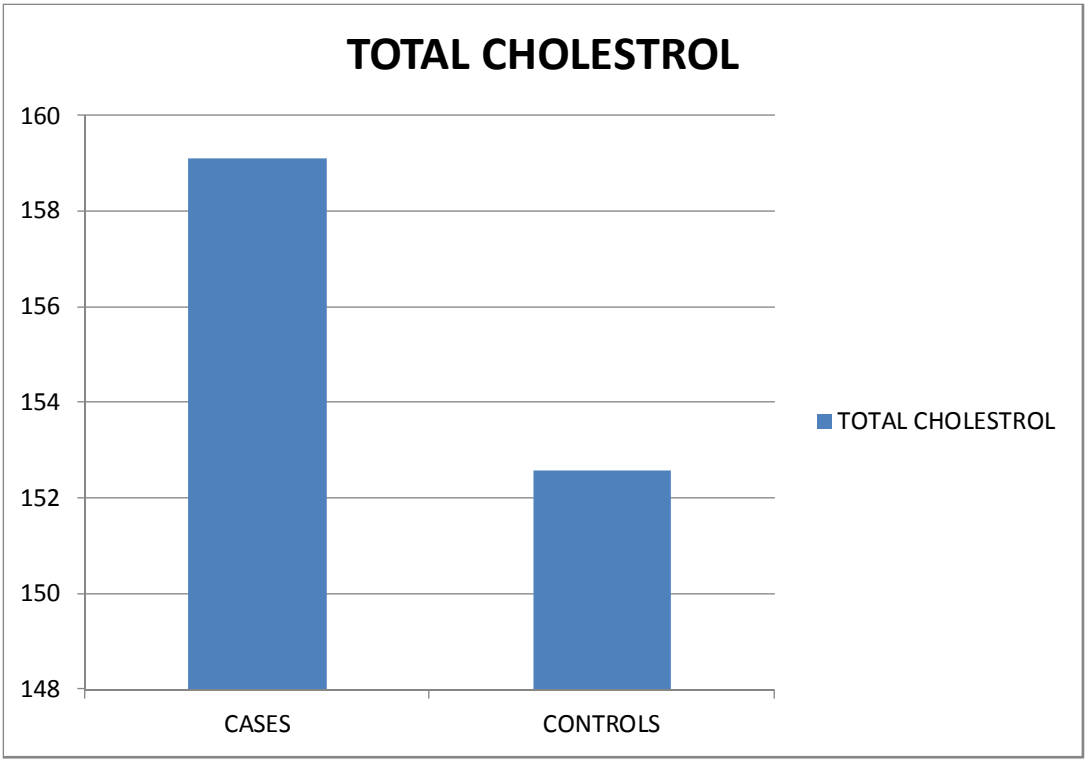
The difference between the two groups with respect to HDL cholestrol is not statistically significant.

TABLE 8 : CASES AND CONTROLS WITH RESPECT TO TOTAL CHOLESTROL

TOTAL CHOLESTROL	Group	No.	Mean	Std. Deviation	Std. Error Mean
	Cases	50	208.98	31.924	4.515
	Control	50	192.20	27.049	3.825

p value – 0.006 (significant)

FIGURE 8 : CASES AND CONTROLS WITH RESPECT TO TOTAL CHOLESTROL



The mean Total Cholestrol among cases and controls is 208.98 and 192.20 mg/dl and a standard deviation of 31.924 and 27.049 respectively,

p value – 0.006.

Inference:

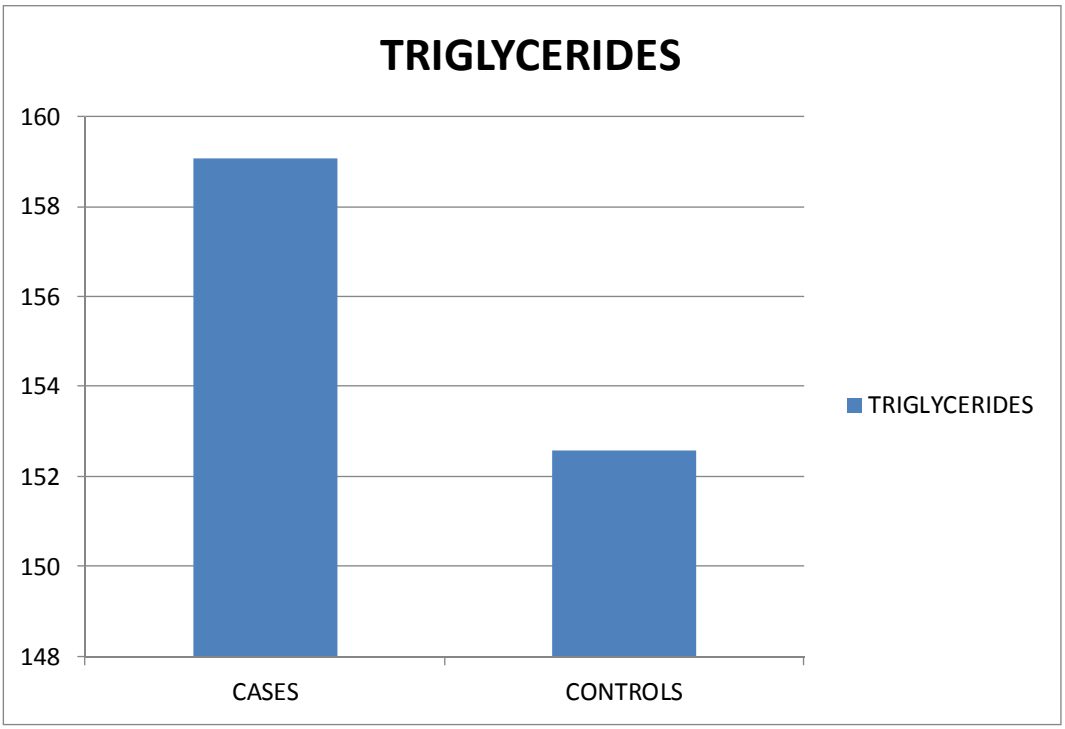
The difference between cases and controls with respect to total cholestrol is statistically significant.

TABLE 9 : CASES AND CONTROLS WITH RESPECT TO TRIGLYCERIDES

TRIGLYCERIDE	Group	No.	Mean	Std. Deviation	Std. Error Mean
	Cases	50	159.10	40.861	5.779
	Control	50	152.58	58.968	8.339

p value – 0.522 (not significant)

FIGURE 9 : CASES AND CONTROLS WITH RESPECT TO TRIGLYCERIDES



The mean Triglyceride level among cases and controls is 159.10 and 152.58 mg/dl and a standard deviation of 40.861 and 58.968 respectively, and p value is 0.522.

Inference:

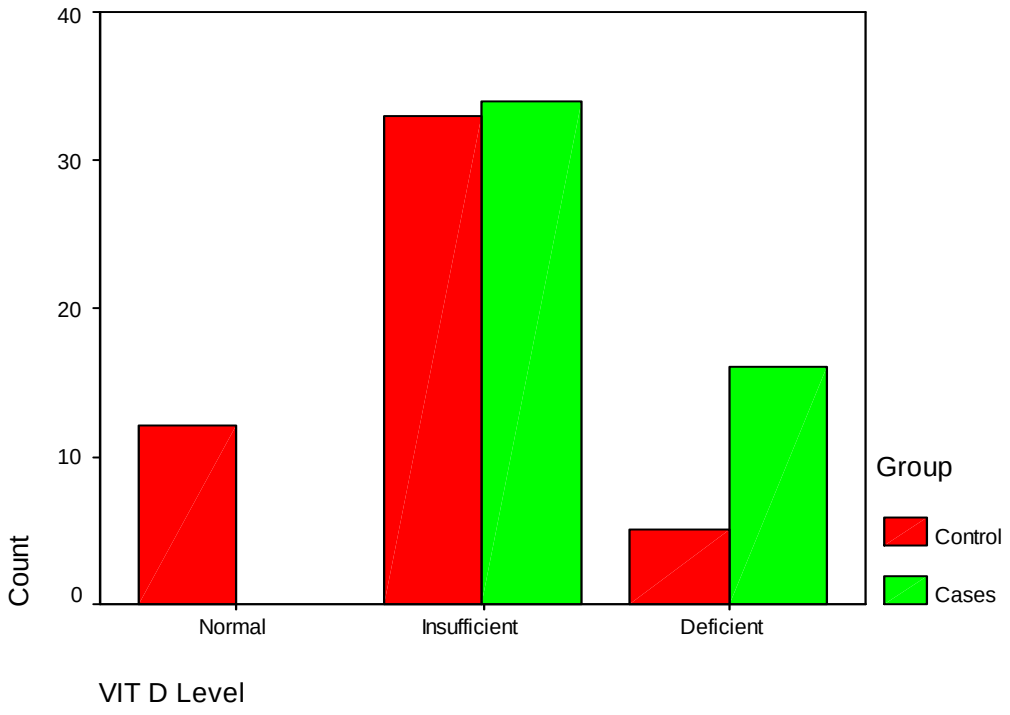
The difference between cases and controls with respect to Triglyceride level is not statistically significant.

TABLE 10 : VITAMIN D STATUS IN THE STUDY POPULATION

			Group		
			Control	Cases	Total
VIT D Level	Normal	Count	12	0	12
		% within VIT D Level	100.0%	.0%	100.0%
		% within Group	24.0%	.0%	12.0%
	Insufficient	Count	33	34	67
		% within VIT D Level	49.3%	50.7%	100.0%
		% within Group	66.0%	68.0%	67.0%
	Deficient	Count	5	16	21
		% within VIT D Level	23.8%	76.2%	100.0%
		% within Group	10.0%	32.0%	21.0%
Total		Count	50	50	100
		% within VIT D Level	50.0%	50.0%	100.0%
		% within Group	100.0%	100.0%	100.0%

p value – 0.001 (highly significant)

FIGURE 10: VITAMIN D STATUS IN THE STUDY POPULATION



Cases:

Vitamin D normal – none

Vitamin D insufficiency – 68%

Vitamin D deficiency – 32%

Controls:

Vitamin D normal – 24%

Vitamin D insufficiency – 66%

Vitamin D deficiency – 10%

The mean serum 25 hydroxy Vitamin D in the cases and controls is

13.48 ± 5.34ng/ml and 23.03 ± 11.28ng/ml respectively with a p value of 0.001.

Inference:

The difference between cases and controls with respect to Vitamin D is statistically highly significant.

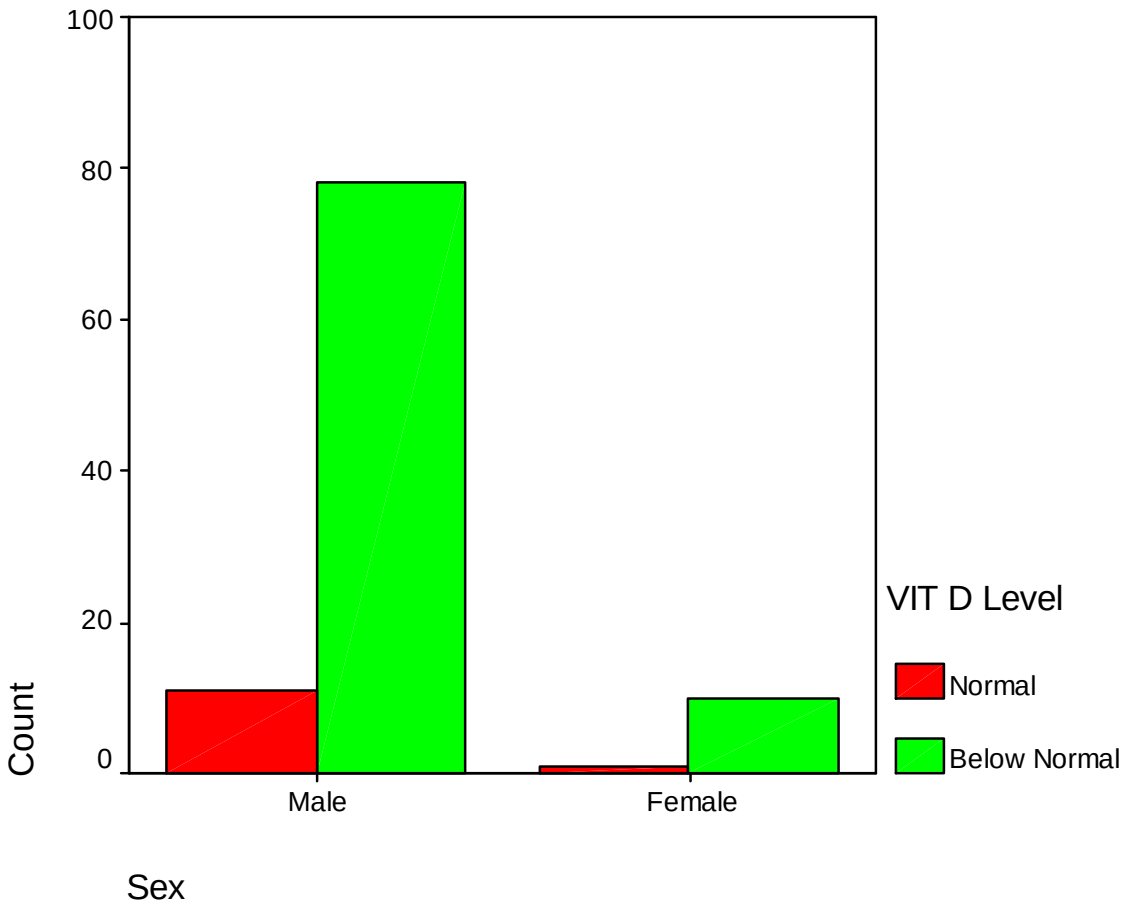
This means that significant hypovitaminosis D is seen among the cases.

TABLE 11: GENDERWISE DISTRIBUTION OF VITAMIN D

			VIT D Level		
			Normal	Below Normal	Total
Sex	Male	Count	11	78	89
		% within Sex	12.4%	87.6%	100.0%
		% within VIT D Level	91.7%	88.6%	89.0%
	Female	Count	1	10	11
		% within Sex	9.1%	90.9%	100.0%
		% within VIT D Level	8.3%	11.4%	11.0%
Total		Count	12	88	100
		% within Sex	12.0%	88.0%	100.0%
		% within VIT D Level	100.0%	100.0%	100.0%

p value – 0.753 (not significant)

FIGURE 11: GENDERWISE DISTRIBUTION OF VITAMIN D



Out of the 89 males included in the study, 11 had normal Vitamin D levels and 78 had below normal levels of Vitamin D.

Out of the 11 females included in the study, 1 had normal Vitamin D level and 10 had below normal levels of Vitamin D.

Inference:

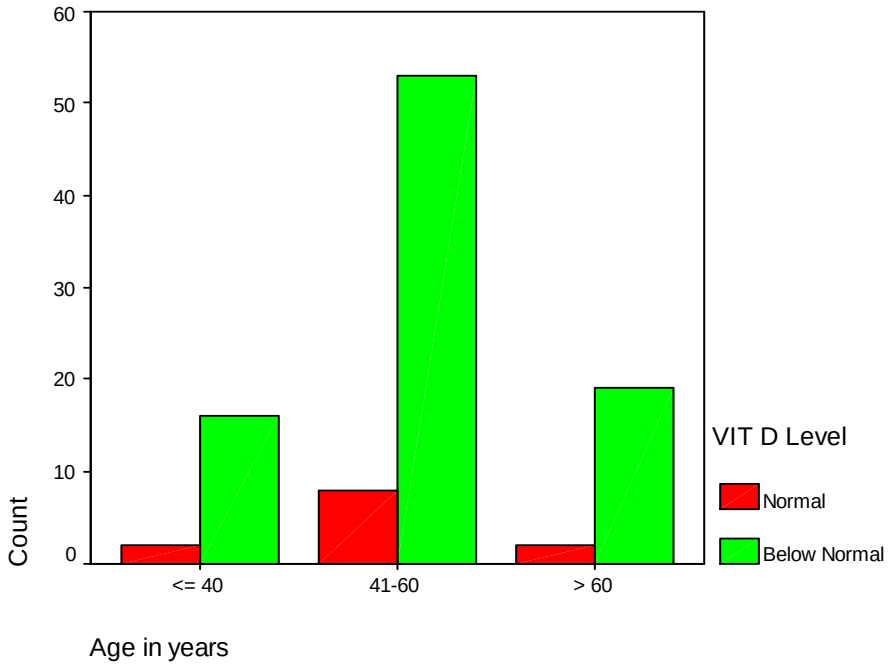
No significant difference in Vitamin D levels between males and females.

TABLE 12: VITAMIN D STATUS IN EACH AGE GROUP

			VIT D Level		
			Normal	Below Normal	Total
Age in years	≤40	Count	2	16	18
		% within Age in years	11.1%	88.9%	100.0%
		% within VIT D Level	16.7%	18.2%	18.0%
	41-60	Count	8	53	61
		% within Age in years	13.1%	86.9%	100.0%
		% within VIT D Level	66.7%	60.2%	61.0%
	> 60	Count	2	19	21
		% within Age in years	9.5%	90.5%	100.0%
		% within VIT D Level	16.7%	21.6%	21.0%
Total		Count	12	88	100
		% within Age in years	12.0%	88.0%	100.0%
		% within VIT D Level	100.0%	100.0%	100.0%

p value – 0.046 (significant)

FIGURE 12: VITAMIN D STATUS IN EACH AGE GROUP



- In the age group 41-60 yrs, 88.9% subjects had below normal levels of Vitamin D.
- In the age group ≤ 40 yrs, 86.9% subjects had below normal levels of Vitamin D.
- In the age group >60 yrs, 90.5% subjects had below normal levels of Vitamin D.
- p value of 0.046

Inference:

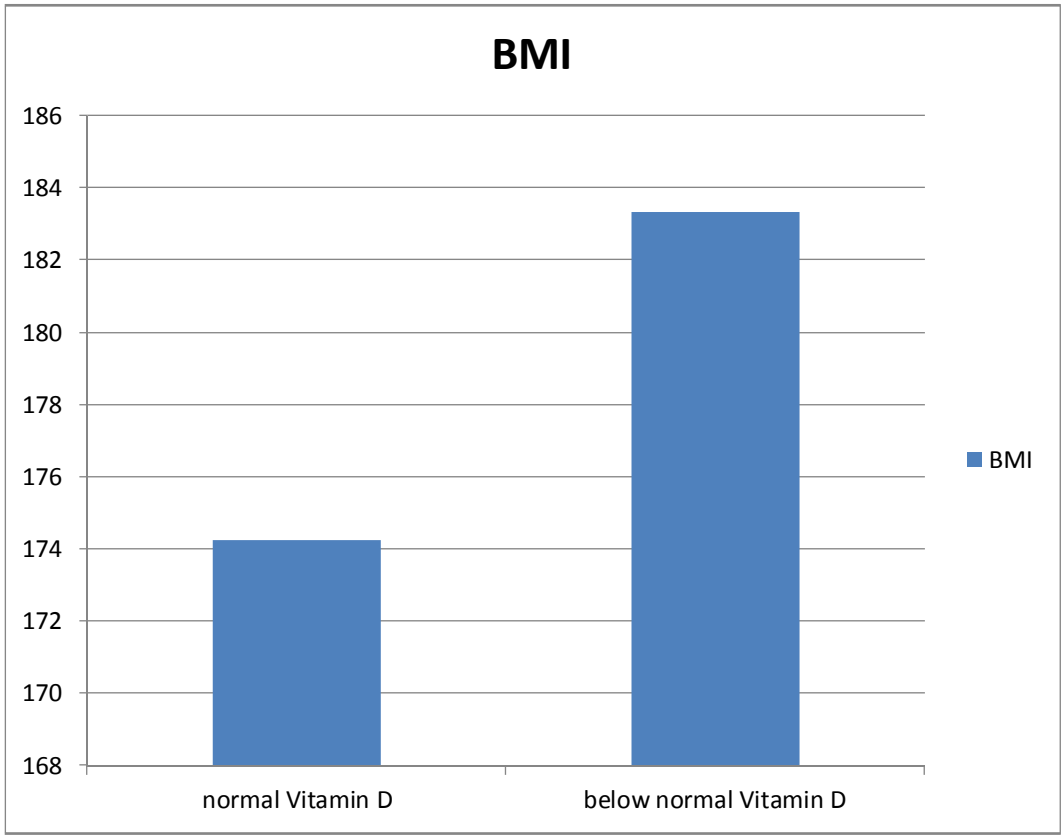
Significant degree of Vitamin D deficiency is seen among all age groups with the level of deficiency being more significant in the cases with age less than 40yrs.

TABLE 13: CORRELATION BETWEEN VITAMIN D STATUS AND BODY MASS INDEX (BMI)

	VIT D Level	No.	Mean	Std. Deviation	Std. Error Mean
BMI	Normal	12	24.2400	2.21329	.63892
	Below Normal	88	25.8528	3.59127	.38283

P value – 0.133 (not significant)

FIGURE 13: CORRELATION BETWEEN VITAMIN D STATUS AND BODY MASS INDEX (BMI)



The mean BMI value among subjects in normal Vitamin D and below normal Vitamin D levels is 24.24 ±2.21 and 25.85 ± 3.59, respectively, with p value of 0.133.

Inference:

- There is no significant difference in BMI between subjects with normal Vitamin D levels and subjects with below normal Vitamin D levels.

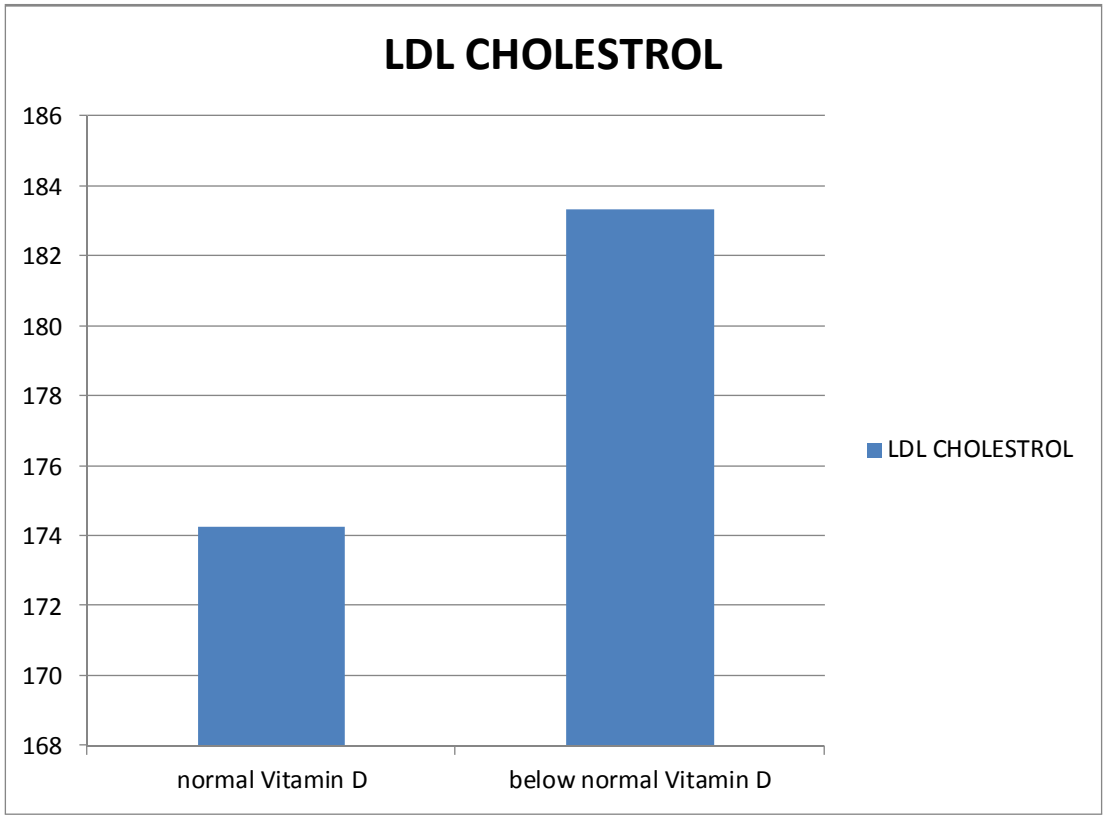
➢ No significant correlation between Vitamin D levels and BMI.

TABLE 14: CORRELATION BETWEEN VITAMIN D STATUS AND LDL CHOLESTROL

	VIT D Level	N	Mean	Std. Deviation	Std. Error Mean
LDL	Normal	12	113.25	27.110	7.826
	Below Normal	88	127.43	32.391	3.453

p value – 0.151 (not significant)

FIGURE 14: CORRELATION BETWEEN VITAMIN D STATUS AND LDL CHOLESTROL



The mean BMI value among subjects in normal Vitamin D and below normal Vitamin D levels is 113.25 ±27.11 and 127.43 ± 32.39, respectively, with p value of 0.151.

Inference:

- There is no significant difference in LDL Cholestrol levels between subjects with normal Vitamin D levels and subjects with below normal Vitamin D levels.

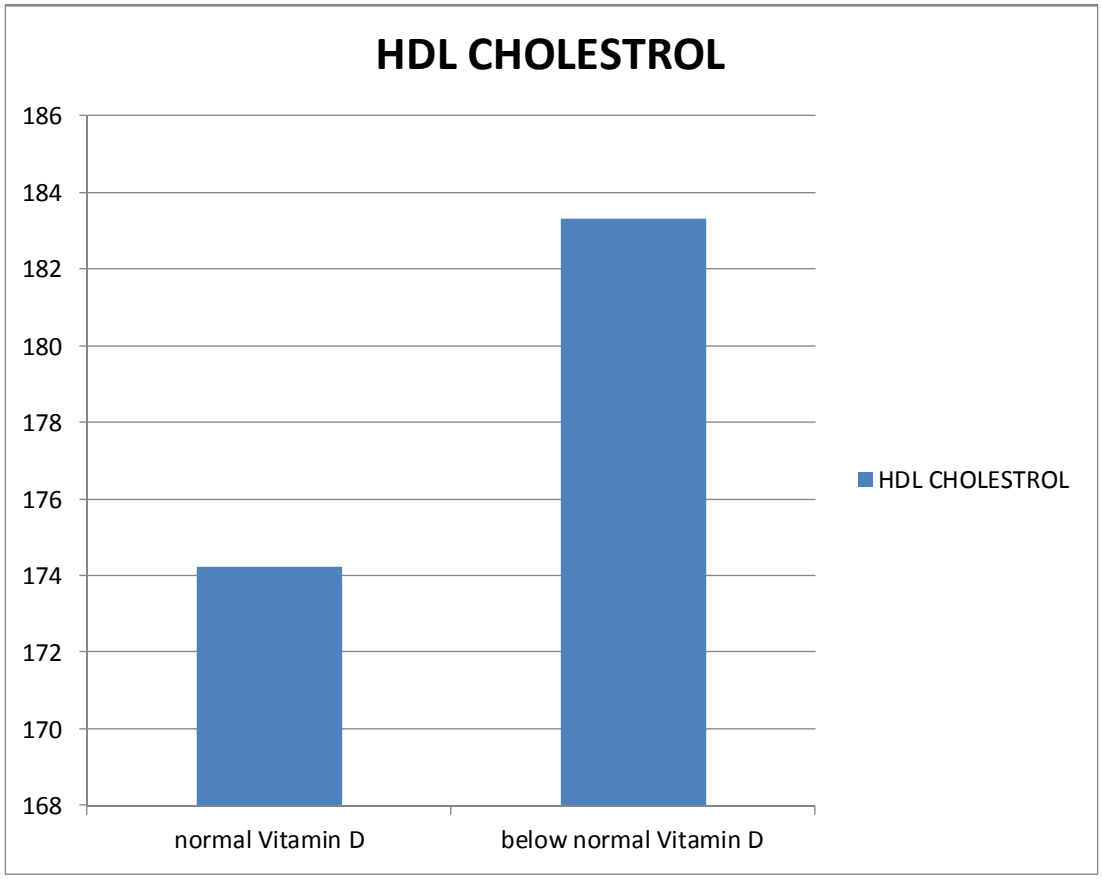
- No significant correlation between Vitamin D levels and LDL Cholestrol.

TABLE 15: CORRELATION BETWEEN VITAMIN D STATUS AND HDL CHOLESTROL

	VIT D Level	N	Mean	Std. Deviation	Std. Error Mean
HDL	Normal	12	45.08	7.255	2.094
	Below Normal	88	43.91	6.149	.655

p value – 0.545 (not significant)

FIGURE 15: CORRELATION BETWEEN VITAMIN D STATUS AND HDL CHOLESTROL



The mean BMI value among subjects in normal Vitamin D and below normal Vitamin D levels is 45.08 ± 7.25 and 43.91 ± 6.14, respectively, with p value of 0.545.

Inference:

- There is no significant difference in HDL Cholestrol levels between subjects with normal Vitamin D levels and subjects with below normal Vitamin D levels.

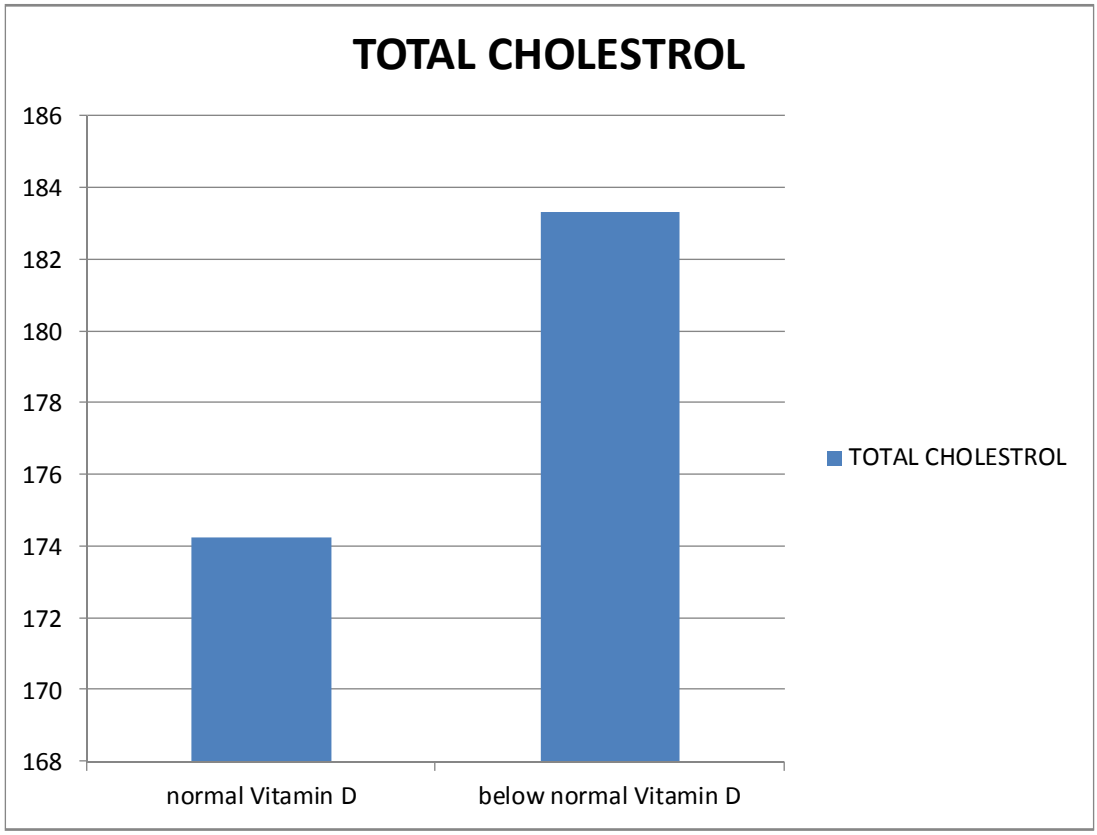
- No significant correlation between Vitamin D levels and HDL Cholestrol.

TABLE 16: CORRELATION BETWEEN VITAMIN D STATUS AND TOTAL CHOLESTROL

	VIT D Level	N	Mean	Std. Deviation	Std. Error Mean
TC	Normal	12	192.83	24.357	7.031
	Below Normal	88	201.65	31.346	3.342

p value – 0.352 (not significant)

FIGURE 16: CORRELATION BETWEEN VITAMIN D STATUS AND TOTAL CHOLESTROL



The mean BMI value among subjects in normal Vitamin D and below normal Vitamin D levels is 192.83 ±24.35 and 201.65 ± 31.34, respectively, with p value of 0.352.

Inference:

- There is no significant difference in Total Cholestrol levels between subjects with normal Vitamin D levels and subjects with below normal Vitamin D levels.

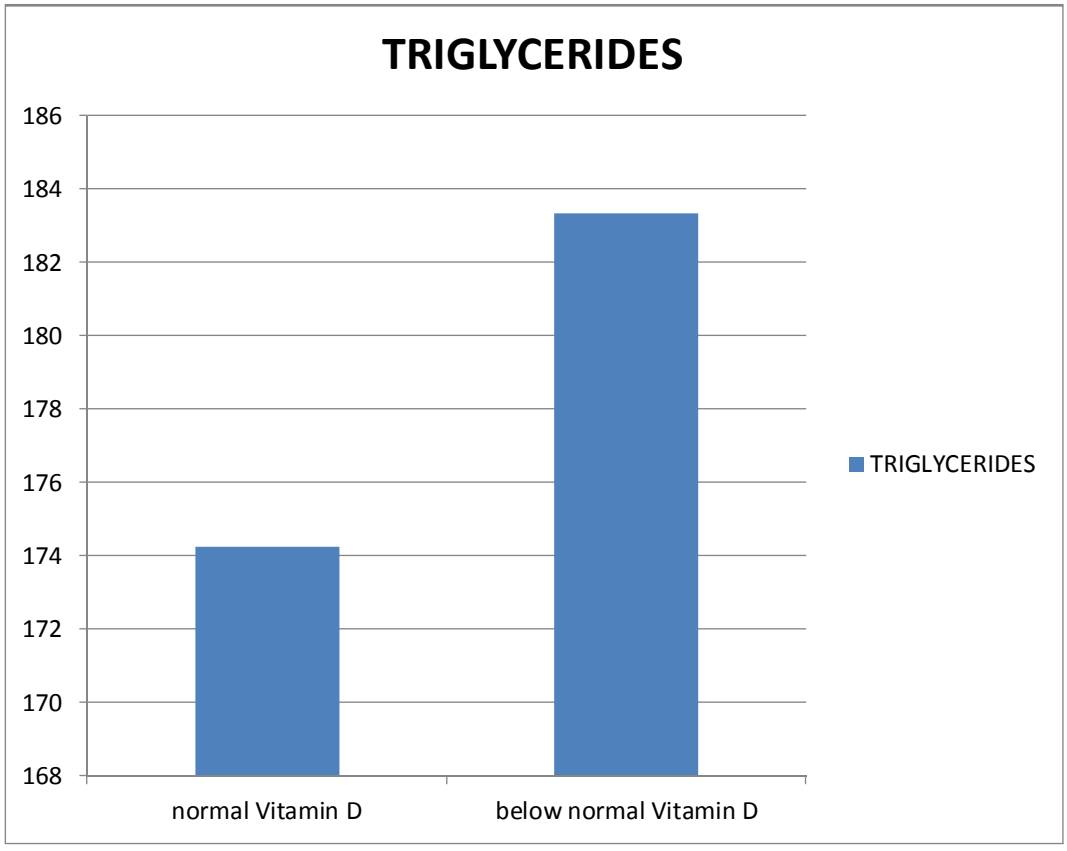
- No significant correlation between Vitamin D levels and Total Cholestrol.

TABLE 17: CORRELATION BETWEEN VITAMIN D STATUS AND TRIGLYCERIDE LEVEL

	VIT D Level	N	Mean	Std. Deviation	Std. Error Mean
Trigly	Normal	12	174.25	72.473	20.921
	Below Normal	88	183.33	46.838	4.993

p value – 0.180 (not significant)

FIGURE 17: CORRELATION BETWEEN VITAMIN D STATUS AND TRIGLYCERIDE LEVEL



The mean BMI value among subjects in normal Vitamin D and below normal Vitamin D levels is 174.25 ± 72.47 and 183.33 ± 46.83, respectively, with p value of 0.180.

Inference:

- There is no significant difference in Triglyceride levels between subjects with normal Vitamin D levels and subjects with below normal Vitamin D levels.

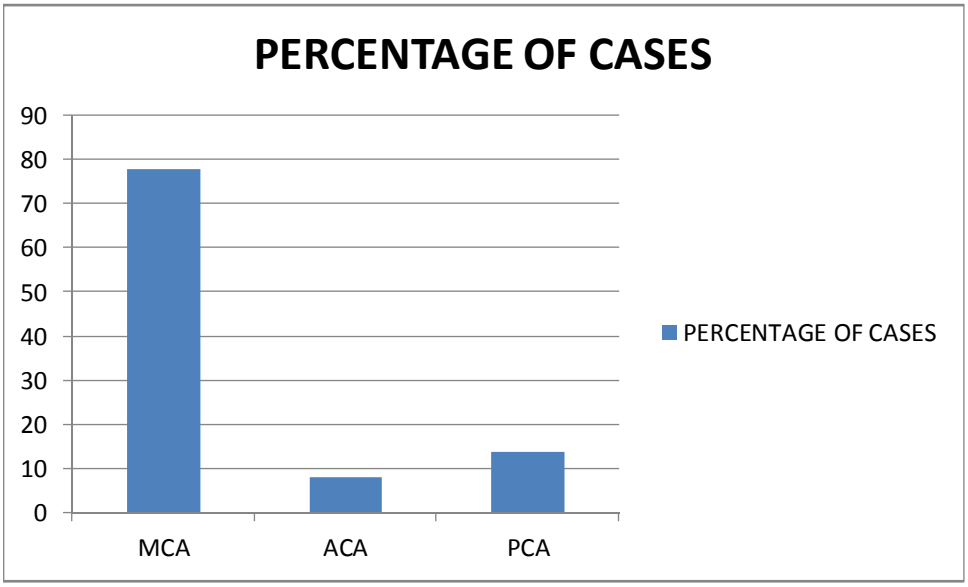
- No significant correlation between Vitamin D and Triglyceride levels.

TABLE 18: CORRELATION BETWEEN VITAMIN D STATUS AND AREA OF INFARCT

			VIT D Level		
			Normal	Below Normal	Total
Area of Infarct	MCA	Count	0	78	78
		% within Area of Infarct		100.0%	100.0%
		% within VIT D Level		78.0%	78.0%
	ACA	Count	0	8	8
		% within Area of Infarct		100.0%	100.0%
		% within VIT D Level		8.0%	8.0%
	PCA	Count	0	14	14
		% within Area of Infarct		100.0%	100.0%
		% within VIT D Level		14.0%	14.0%
Total		Count	0	100	100
		% within Area of Infarct		100.0%	100.0%
		% within VIT D Level		100.0%	100.0%

p value – 0.049 (significant)

FIGURE 18: CORRELATION BETWEEN VITAMIN D STATUS AND TERRITORY (AREA) OF INFARCT



Among all the cases, 78% had MCA infarct, 8% had ACA infarct and 14% had PCA infarct.

p value – 0.049 (significant)

Inference:

There is significant correlation between Vitamin D levels and territory (area) of infarct.

Cases with below normal Vitamin D levels have infarcts involving the MCA territory mainly.

DISCUSSION

DISCUSSION

In our study of 100 patients, 50 cases with acute ischaemic stroke and 50 age and sex matched controls were included. We had selected our cases in such a way that they had no history of systemic hypertension, diabetes mellitus, chronic kidney disease, coronary artery disease or prior stroke. This was done to study whether low Vitamin D levels could be an independent risk factor for the occurrence of cerebro vascular accident in patients.

VITAMIN D AND STROKE

We found a **statistically significant correlation between below normal Vitamin D levels and Acute Ischaemic Stroke**. All the patients who were admitted with acute ischaemic stroke had below normal Vitamin D levels. Vitamin D deficiency (<10ng/ml) was seen in 32% of the cases and 26% of the controls. Among the controls, 10% had Vitamin D deficiency, 66% had Vitamin D insufficiency and only 24% had normal Vitamin D levels. The mean Vitamin D levels among cases and controls was 13.48 and 23.03ng/ml respectively with a statistically significant difference.

Study done by **Tu WJ et al**[51] showed a Vitamin D level of 10-18ng/ml among stroke cases and 17-22ng/ml among controls. **Kenneth et al**[49] found 77% prevalence of Vitamin D deficiency among stroke patients whereas the prevalence is 100% in our study.

Studies done by **Pilz et al[50], de Silva et al[55] and LURIC study[53]** showed a Vitamin D deficiency in 58%, 95% and 92% respectively among stroke patients.

On assessing the overall study population of 100 subjects, we found only 12% of the subjects had normal Vitamin D levels. The remaining 88% had below normal Vitamin D levels with 21% having Vitamin D deficiency and 67% Vitamin D insufficiency. This high rate of Vitamin D deficiency in our study group reflects the high prevalence of Vitamin D deficiency in Indian population as already demonstrated by **Harinarayan et al[39]** showing prevalence of Vitamin D deficiency to be around 50-90% among Indian population.[62]

The control group showed a mean Vitamin D level of 23.03ng/ml. This is similar to the mean value of 24ng/ml among apparently healthy subjects shown by the **NHANES 2005-2006 survey**[54].

In our study, the **association between ischaemic stroke and parameters** like Gender, Age, Body Mass Index, Smoking, LDL cholestrol, HDL cholestrol, Total cholestrol, Triglycerides and Area of infarct was also analysed. Apart from Body Mass Index, statistical significance was not found with any other parameter. Hence we could safely exclude most of these confounding factors, which are themselves independent risk factors of stroke.

The **association between Vitamin D and parameters** like Gender, Age, Body Mass Index, Smoking, LDL cholestrol, HDL cholestrol, Total cholestrol, Triglycerides and Area of infarct was also analysed in our study.

VITAMIN D AND GENDER VARIATION

We did not find any significant association between Vitamin D levels and **gender distribution**. Normal Vitamin D levels were found in 12% of males and 9% of females. 88% of males and 91% of females had below normal Vitamin D levels. This is contrary to the study done by **Johnson et al**[47] which showed significant Vitamin D deficiency among men than women.

VITAMIN D AND AGE DISTRIBUTION

The study population was divided into 3 **age groups** (<40yrs, 41-60yrs, >60yrs). Below normal Vitamin D levels were found in the three age groups were 89%, 87% and 90% respectively. There was significant hypovitaminosis D in the young stroke patients (<40yrs of age). This showed Vitamin D deficiency as an independent risk factor for young stroke in the absence of other conventional risk factors. On the contrary, **Harinarayan et al**[39] didnot find any significant difference among various age groups.

VITAMIN D AND SMOKING

There was no significant association between Vitamin D and smoking in our study. But a study done by **Eugenia Cutillas et al**[48] had shown significant Vitamin D deficiency in smokers.

VITAMIN D AND OBESITY

Body Mass Index was found to higher in subjects with below normal Vitamin D levels. Body mass index >25kg/m^2 was seen in approximately 70% of the subjects with Vitamin D deficiency. But high BMI values were not statistically significant. This is not in concurrence with many studies that link high BMI(obesity) to Vitamin D deficiency. Obesity can itself lead to atherosclerosis and cause stroke. Thus, our study showed association between Vitamin D deficiency and stroke, without significant effect of Vitamin D on Body Mass Index. Studies done by **Calin et al** and **Aasheim et al**[48] had shown people with Vitamin D deficiency having high BMI values.

VITAMIN D AND DYSLIPIDEMIA

We did not find any association between Vitamin D and dyslipidemia (LDL cholestrol, HDL cholestrol, Total cholestrol, Triglycerides). But studies done by **Chaudhuri et al**[71] and **Zittermann et al**[72] shown dyslipidemia in patients with Vitamin D deficiency.

VITAMIN D AND TERRITORY OF INFARCT

Association between Vitamin D deficiency and territory of infarct was significant (p-0.049) with most of the cases with Vitamin D deficiency having MCA territory infarct. We could not get any previous study relating the territory of infarct to Vitamin D deficiency. Probably our study is one of the first few studies to identify the correlation between Vitamin D deficiency and area of infarct.

CONCLUSION

CONCLUSION

- A wide **variety of diseases** are associated with Vitamin D deficiency. This poses a great burden on the community. Hence, the discovery of the causal association of Vitamin D deficiency in stroke is indeed an important breakthrough.
- In our study, ischaemic stroke patients had significantly low levels of Vitamin D.
- There is growing body of evidence suggesting low Vitamin D levels may be a **potentially modifiable cerebrovascular risk factor**. Our results provide additional evidence to this.
- These results may have broad public health implications due to the high prevalence of Vitamin D deficiency in our country.
- Vitamin D deficiency is an **easily measurable and correctable** risk factor. The treatment is cost effective and safe. Therefore, general awareness needs to be created about this risk factor and guidelines need to be implemented regarding its diagnosis and treatment.

- **Adequate sun exposure and food fortification** are simple preventive measures which can bring about a significant reduction in cerebrovascular events and other diseases associated with Vitamin D deficiency.
- **Large scale randomized control trials** are needed to further analyse Vitamin D deficiency as a risk factor for stroke and the effects of Vitamin D supplementation on the prevention of stroke.

LIMITATIONS

OF

THE

STUDY

LIMITATIONS OF THE STUDY

- The main limitation in our study is the **small number of subjects** included in the study.
- Female patients included in the study were very few in number. Further studies need to be done including larger number of women.
- Parathormone levels were not measured. Hence its role as a confounding factor could not be analysed.
- The **cause** for the below normal Vitamin D in the subjects was not evaluated.
- We could not assess the prognosis of the patients after Vitamin D supplementation as they were not followed up.
- The etiology for ischaemic stroke in the young patients was not studied and so we could not rule our other possible confounding factors.

IMPLICATIONS

FOR THE

FUTURE

IMPLICATIONS FOR THE FUTURE

- Measurement of serum 25 hydroxy Vitamin D levels should be **routinely done** in all patients with Diabetes mellitus, systemic hypertension, dyslipidemia and other stroke risk factors because early detection and correction of deficiency states has proven beneficial effects in preventing the occurence of stroke.

- Estimation of Vitamin D levels in the general population could help identify low normal levels and correct the deficit, so that, conditions like diabetes mellitus, systemic hypertension, coronary heart disease and ischaemic stroke can be prevented. Further large scale studies are needed to firmly implement the need to measure Viatmin D levels in the general population.

- India is a developing country with high incidence of stroke in urban and rural population where poverty and illeteracy dominate. Therefore public health awareness programs and food fortification programs need to be implemented to overcome this deficit.

- **Vitamin D - Food fortification programs** is the need of the hour in our country. Many developed countries have already initiated fortification of foods such as milk and dairy products with Vitamin D. This is a very efficient method to reduce the incidence of Vitamin D deficiency mainly in people on a pure vegetarian diet.

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ANNEXURES

ABBREVIATIONS

rt-PA	- recombinant tissue plasminogen activator
WHO	- World Health Organisation
ICMR	- Indian Council of Medical Research
RDA	- Recommended Daily Allowance
LDL	- Low Density Lipoprotein
HDL	- High Density Lipoprotein
TGL	- Triglycerides
TC	- Total Cholestrol
BMD	- Bone Mineral Density
GCS	- Glascow Coma Scale
25(OH)D	- 25 hydroxy Vitamin D
TGF	- Transforming Growth Factor
UVB	- Ultraviolet B
PTH	- Parathormone
NCD	- Non Communicable Disease
TNF	- Tumor Necrosis Factor

DATA COLLECTION FORM (PROFORMA)

NAME:

AGE:

SEX:

OCCUPATION:

ADDRESS:

IP.No.:

DATE OF ADMISSION:

DATE OF EVENT OF STROKE:

H/O Smoking:

Height (m):

Weight (kg):

BMI (kg/m^2):

LDL cholestrol:

HDL cholestrol:

Triglycerides:

Total Cholestrol:

Serum Vitamin D:

CT BRAIN:

(area of infarct)

PATIENT CONSENT FORM

STUDY DETAIL:

STUDY CENTRE:

PATIENT'S NAME:

PATIENT'S AGE:

IDENTIFICATION NUMBER:

I confirm that I have understood the purpose and procedure of the above study. I have the opportunity to ask questions and all my questions and doubts have been answered to my complete satisfaction.

I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving reason, without my legal rights being affected.

I understand that the sponsor of the clinical study, others working on the sponsor's behalf, the ethical committee and the regulatory authorities will not need my permission to look at my health records, both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the study I agree to this access. However I understand that my identity would not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from this study.

I hereby consent to participate in this study.

I hereby give permission to undergo complete clinical examination and diagnostic tests including haematological, biochemical, radiological tests.

Signature/thumb impression:

Patient's name and address: Place: Date:

Signature of the investigator:

Name of the investigator: Place: Date:

நோயாளி ஒப்புதல் படிவம்

ஆராய்ச்சியின் விவரம்: குருதி ஊட்டக்குறை பக்கவாத நோயாளிகளின் இரத்த நீர்த்த பித்தச்செம்பசை அளவு (சீரம் பில்லிரூபின்) மற்றும் அவற்றின் தொடர்பினை ஆராய்யும் ஆய்வரிக்கை.

ஆராய்ச்சி மையம்: அரசு கீழ்பாக்கம் மருத்துவக் கல்லூரி மருத்துவமனை

நோயாளியின் பெயர்: நோயாளியின் வயது:

பதிவு எண்:

நோயாளி கீழ்கண்டவற்றுள் கட்டங்களை (✓) செய்யவும்

1.	மேற்குறிப்பிட்டுள்ள ஆராய்ச்சியின் நோக்கத்தையும் பயனையும் முழுவதுமாக புரிந்துகொண்டேன். மேலும் எனது அனைத்து சந்தேகங்களையும் கேட்டு அதற்கான விளக்கங்களையும் தெளிவுபடுத்திக் கொண்டேன்.	
2.	மேலும் இந்த ஆராய்ச்சிக்கு எனது சொந்த விருப்பத்தின் பேரில் பங்கேற்கிறேன் என்றும், மேலும் எந்த நேரத்திலும் எவ்வித முன்னறிவிப்புமின்றி இந்த ஆராய்ச்சியிலிருந்து விலக முழுமையான உரிமை உள்ளதையும், இதற்கு எவ்வித சட்ட பிணைப்பும் இல்லை என்பதையும் அறிவேன்.	
3.	ஆராய்ச்சியாளரோ, ஆராய்ச்சி உதவியாளரோ, ஆராய்ச்சி உபயத்தாரரோ, ஆராய்ச்சி பேராசிரியரோ, ஒழுங்குநெறி செயற்குழு உறுப்பினர்களோ எப்போது வேண்டுமானாலும் எனது அனுமதியின்றி எனது உள்நோயாளி பதிவுகளை இந்த ஆராய்ச்சிக்காகவோ அல்லது எதிர்கால பிற ஆராய்ச்சிகளுக்காகவோ பயன்படுத்திக்கொள்ளலாம் என்றும் மேலும் இந்த நிபந்தனை நான் இவ்வராய்ச்சியிலிருந்து விலகினாலும் தகும் என்றும் ஒப்புக்கொள்கிறேன். ஆயினும் எனது அடையாளம் சம்பந்தப்பட்ட எந்த பதிவுகளும் (சட்டபூர்வமான தேவைகள் தவிர) வெளியிடப்படமாட்டது என்ற உறுதிமொழியின் பெயரில் இந்த ஆராய்ச்சியிலிருந்து கிடைக்கப்பெறும் முடிவுகளை வெளியிட மறுப்பு தெறிவிக்கமாட்டேன் என்று உறுதியளிக்கின்றேன்.	
4.	இந்த ஆராய்ச்சிக்கு நான் முழுமனதுடன் சம்மதிக்கின்றேன் என்றும் மேலும் ஆராய்ச்சிக் குழுவினர் எனக்கு அளிக்கும் அறிவுரைகளை தவறாது பின்பற்றுவேன் என்றும் உறுதியளிக்கின்றேன்.	
5.	இந்த ஆராய்ச்சிக்குத் தேவைப்படும் அனைத்து மருத்துவப் பரிசோதனைகளுக்கும் ஒத்துழைப்பு தருவேன் என்று உறுதியளிக்கின்றேன்.	
6.	இந்த ஆராய்ச்சிக்கு யாருடைய வற்புறுத்தலுமின்றி எனது சொந்த விருப்பத்தின் பேரிலும் சுயஅறிவுடனும் முழுமனதுடனும் சம்மத்திக்கின்றேன் என்று இதன் மூலம் ஒப்புக்கொள்கிறேன்.	

நோயாளியின் கையொப்பம் / பெருவிரல் கைரேகை:

இடம்: தேதி:

ஆராய்ச்சியாளரின் கையொப்பம்:

இடம்: தேதி:

MASTER CHART

CASES

S.No.	NAME	AGE	SEX	BMI	SMOKING	LDL	HDL	TRIGLY	TC	VIT D LEVEL	AREA OF INFARCT
1	Jeyakumar	26	M	31.24	Y	111	46	169	190	10.09	MCA
2	Selvaraj	74	M	26.67	N	129	42	109	192	27.39	MCA
3	Suresh	27	M	21.34	Y	129	38	165	200	8.26	PCA
4	Arumugam	57	M	24.91	Y	101	45	135	173	19.32	MCA
5	Sahul Hameed	46	M	24.77	Y	91	52	110	165	11.88	MCA
6	Deenadayalan	60	M	30.52	Y	99	47	135	173	7.28	PCA
7	Dorairaj	58	M	24.69	Y	92	40	96	151	18.2	MCA
8	Ravi	53	M	28.85	Y	132	37	115	192	11.08	MCA
9	Palani	43	M	21.26	N	91	39	160	162	28.32	MCA
10	Chelladurai	33	M	23'05	Y	181	34	154	245	8.94	MCA
11	Sambantham	59	M	29.43	Y	158	38	120	220	14.86	MCA
12	Sriramulu	70	M	29.52	Y	223	30	110	275	21.71	MCA
13	Mary	60	F	29.3	N	209	47	174	290	14.1	MCA
14	Arivudainambi	43	M	30.78	Y	219	41	127	285	10.35	PCA
15	Ismail	42	M	31.56	Y	150	43	87	210	7.91	MCA
16	Maria	65	F	32.89	N	154	49	210	245	12.21	MCA
17	Palanivel	60	M	27.67	Y	132	44	198	215	9.68	MCA
18	Swaminathan	62	M	29.9	N	128	51	201	219	24.39	MCA
19	Subramani	80	M	20.05	N	116	49	150	195	14.08	MCA
20	Babu	42	M	34.5	Y	106	47	135	180	15.96	ACA
21	Rajkumar	56	M	31.87	Y	94	51	135	172	7.93	MCA
22	Jeganathan	43	M	30.08	Y	186	37	208	264	15.77	PCA
23	Chandran	65	M	23.34	N	112	48	163	192	16.2	MCA
24	Iqbal Basha	52	M	27.56	Y	92	46	140	166	18.12	MCA
25	Kathirvel	50	M	27.81	Y	125	41	158	197	10.92	MCA
26	Sekar	67	M	24.52	Y	125	49	183	210	7.6	MCA
27	Lakshmi	70	F	29.9	N	103	56	148	188	9.23	MCA
28	Paramasivam	53	M	28.03	Y	111	47	145	187	13.87	MCA
29	Palani	62	M	21.89	Y	114	39	200	193	12.11	MCA
30	Muthukumar	49	M	32.15	Y	163	49	136	239	6.57	ACA
31	Velu	30	M	27.82	Y	171	38	128	234	10.97	PCA
32	Sekar	45	M	27.8	Y	171	41	136	239	7.19	MCA
33	Dhanapal	67	M	25.92	Y	134	43	109	198	13.15	MCA
34	Koti	56	M	30.05	Y	94	50	139	171	9.38	MCA
35	Saif	54	M	22.87	Y	141	36	246	226	20.6	MCA
36	Balaji	36	M	20.14	Y	120	39	236	206	10.3	MCA
37	Perumal	58	M	22.43	Y	131	43	237	221	15.76	PCA
38	Mala	62	F	24.78	N	108	53	158	192	15.1	MCA
39	Sahira Banu	42	F	25.5	Y	102	57	140	187	9.79	MCA
40	Kali	29	M	23.73	Y	123	42	225	210	7.62	MCA
41	Babu	42	M	24.19	Y	117	47	106	185	11.23	MCA
42	Md.Talip	45	M	20.95	Y	142	44	124	210	16.17	MCA
43	Basha	35	M	24.91	N	140	51	145	220	20.14	PCA
44	Vincent	63	M	23.5	Y	148	39	228	232	18.94	MCA
45	Murugan	74	M	28.73	Y	168	37	187	242	10.03	PCA
46	Srikanth	59	M	32.3	N	153	45	204	238	9.56	MCA
47	Dhanasekar	46	M	23.17	Y	145	41	224	230	21.09	MCA
48	Munusamy	37	M	25.67	N	151	49	198	239	15.43	ACA
49	Hariharan	41	M	29.78	Y	118	52	149	199	8.47	MCA
50	Pratap	51	M	26.56	Y	110	43	160	185	9.22	MCA

CONTROLS

S.No.	NAME	AGE	SEX	BMI	SMOKING	LDL	HDL	TRIGLY	TC	VIT D LEVEL
1	Nataraj	67	M	25.09	N	93	44	105	158	16.47
2	Ravikumar	40	M	23.07	Y	93	48	111	163	20.02
3	araman	40	M	23.45	Y	123	41	93	182	30.8
4	Ganesh	35	M	24.15	Y	106	46	103	172	23.29
5	Sivaraman	46	M	26.89	Y	143	53	144	224	13.41
6	Govindsamy	52	M	22.51	Y	130	38	110	190	49.23
7	Kandamani	60	F	22.92	N	150	49	101	219	15.17
8	Mani	65	M	23.34	N	107	50	138	184	25.26
9	Pachiannan	55	M	19.87	Y	99	47	96	165	27.38
10	Moorthy	42	M	21.34	Y	108	38	119	169	9.18
11	Chandran	35	M	24.22	N	123	31	112	176	20.7
12	Sardar	55	M	20,08	Y	106	45	105	172	19.77
13	Abdul	60	M	30.87	Y	200	37	99	256	15.2
14	Bhaskar	52	M	25.56	N	138	41	117	202	33.06
15	Ellappan	74	M	27.8	N	103	53	98	175	35.43
16	kumar	52	M	29.49	Y	101	49	92	168	10.03
17	Pandian	55	M	30.21	Y	112	43	93	173	13.23
18	Sivakumar	52	M	27.68	Y	129	47	120	200	9.83
19	Mani	52	M	26.22	N	109	46	122	179	13.19
20	Kanniappan	48	M	21.34	Y	150	39	103	209	18.66
21	Muthusamy	75	M	23.8	N	102	41	108	164	23.37
22	Mariappan	60	M	24.49	Y	91	45	223	180	11.5
23	Gangaiyan	70	M	21.37	N	138	38	235	223	19.66
24	Vasu	40	M	26.67	Y	125	40	135	192	20.17
25	Manohar	48	M	27.93	Y	179	32	251	261	43.62
26	Srinivas	46	M	22.92	Y	135	35	142	198	10.15
27	Meena	51	F	24.56	N	90	54	213	186	16.06
28	Subramani	58	M	19.87	Y	115	37	139	179	10.79
29	Meera	52	F	23.47	N	91	51	246	191	8.22
30	James	52	M	21.9	Y	91	42	222	177	51.7
31	Vanaraj	60	M	23.5	Y	111	35	124	170	8.56
32	Kamaraj	34	M	22.89	Y	133	41	154	204	29.33
33	Pichai	70	M	21.16	Y	83	50	240	181	36.78
34	Manickam	55	M	20.86	Y	145	39	170	218	28.14
35	Surendar	42	F	25.54	Y	71	56	230	173	28.5
36	Sarada	62	M	22.68	Y	82	49	105	152	18.78
37	Ponraj	48	M	22.71	Y	94	51	115	168	34.89
38	Raman	45	M	23.34	Y	92	47	96	158	26.73
39	Latha	50	F	26.12	Y	96	58	282	210	41.7
40	Ganesh	55	M	23.53	Y	122	41	123	187	35.6
41	Dilli Babu	60	M	28.12	Y	123	43	274	220	12.8
42	Chinnasamy	62	M	30.09	Y	197	35	150	262	20.93
43	Kolandaivel	51	M	25.2	Y	160	33	110	215	23.45
44	Kalyan	48	M	24.76	N	109	48	178	192	39.88
45	Vijaykumar	34	M	23.45	Y	91	46	262	189	42.56
46	Noor	40	F	24.9	N	140	57	159	228	21.67
47	Sampath	56	M	26.57	Y	166	39	128	230	8.93
48	Shankar	30	M	28.85	Y	143	36	235	226	16.72
49	Jayavendan	59	M	20.43	Y	103	41	140	172	19.87
50	Rajesh	40	M	27.3	N	69	48	259	168	21.31

INSTITUTIONAL ETHICAL COMMITTEE
GOVT. KILPAUK MEDICAL COLLEGE,
CHENNAI-10

Ref.No.134/ME-1/Ethics/2014 Dt:06.02.2014

CERTIFICATE OF APPROVAL

The Institutional Ethical Committee of Govt. Kilpauk Medical College, Chennai reviewed and discussed the application for approval "A Study on serum vitamin D levels in acute ischaemic stroke" – For Project Work submitted by Dr.S.Bala Vignesh, MD(GM), PG Student, KMC, Chennai-10.

The Proposal is APPROVED.

The Institutional Ethical Committee expects to be informed about the progress of the study any Adverse Drug Reaction Occurring in the Course of the study any change in the protocol and patient information /informed consent and asks to be provided a copy of the final report.

26/3/14

CHAIRMAN,
Ethical Committee
Govt.Kilpauk Medical College,Chennai

GOVT. KILPAUK MEDICAL COLLEGE
26 MAR 2014
Kilpauk, Chennai-10.